WORLD HEALTH ORGANIZATION

TECHNICAL REPORT SERIES

No. 399

MICROBIOLOGICAL ASPECTS OF

FOOD HYGIENE

Report of a WHO Expert Committee
with the participation of FAO

WORLD HEALTH ORGANIZATION

GENEVA

1968

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WHO EXPERT COMMITTEE ON THE MICROBIOLOGICAL ASPECTS OF FOOD HYGIENE

with the participation of FAO

Geneva, 10 - 16 October 1967

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WHO EXPERT COMMITTEE ON THE MICROBIOLOGICAL ASPECTS OF FOOD HYGIENE

with the participation of FAO

Report

A WHO Expert Committee on the Microbiological Aspects of Food Hygiene met in Geneva from 10 to 16 October 1967, FAO having participated in its planning and preparation. Professor Aage Jepsen was elected Chairman, Dr Betty Hobbs and Dr J. Takács Vice-Chairmen, and Dr E. H. Kampelmacher Rapporteur.

INTRODUCTION

The Committee was primarily concerned with assessing the role of the microbiological laboratory within the over-all programme for the promotion and control of food hygiene. In recent years the laboratory has been making an increasing contribution to food hygiene programmes, and it is assuming a key role in diagnosis, surveillance, control, and research. The Committee was asked to consider the effective integration of the laboratory within the total effort to achieve safe food ; the agenda were, therefore, extensive, and included, in addition to the areas noted above, the contributions of the laboratory to food hygiene education, training, and technology.

Specifically, the Committee discussed microbiological criteria for foods, the interpretation of laboratory results, the use of such results by administrators of food hygiene programmes, the effective use of research findings in food hygiene programmes, and the need for future research. The Committee also dealt with the role of the laboratory in surveillance for potential sources of food-borne disease and the need for co-operation among laboratories. The development of microbiological standards was not undertaken, since it was considered to be a task for special groups convened for the purpose.

The past activities of WHO, FAO, and other international organizations in the field of food hygiene have consisted principally of the provision of advisory services to countries requesting such assistance and of the issuing of various publications.[1]

The Inter-Agency Working Group on Milk and Milk Products, which collaborates with the International Dairy Federation and other interested bodies, has assisted the development of rural dairies in Africa and Asia, studied the economic effects of dairy development in developing countries, and provided dairy education and training courses.

A WHO/FAO Seminar on Zoonoses was held in Vienna in 1952, a WHO/FAO Seminar on Meat Hygiene in Copenhagen in 1954, a European Seminar on Veterinary Public Health in Warsaw in 1957, and a European Symposium on Collaboration between Veterinary Services and Public Health Services in Ghent in 1966. In view of the danger that diseases might be spread through traffic in animals and food, a joint FAO/OIE/WHO Meeting on Basic Principles for the Control of International Traffic of Animals and Animal Products was held in Bern in 1964. Numerous courses, seminars, and other meetings on food hygiene have also been held by WHO, FAO, and other international organizations.

An important development is the Joint FAO/WHO Food Standards Programme, whose principal responsibility is to prepare the Codex Alimentarius, a collection of internationally-adopted standards for all the principal foods, whether processed, semiprocessed, or raw. The Codex Alimentarius includes provisions related to food hygiene, food additives, pesticide residues, contaminants, labelling and presentation, and methods of analysis and sampling, all aimed at protecting the health of the consumer and ensuring fair practices in the food trade. The food hygiene aspects of this work have been relegated to a special committee, the Codex Alimentarius Committee on Food Hygiene ; this committee works in close collaboration with the FAO and WHO secretariats.

1. REPORTING AND SURVEILLANCE

In recent years there has been extensive discussion of the epidemiology of food-borne disease, and a number of FAO and WHO publications have considered the subject both generally [2] and with specific reference to milk

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1951, **40** ; 1955, **99** ; 1957, **124** ; 1959, **169**, **184** ; 1960, **197** ; 1962, **241** ; 1967, **378** (*FAO Agricultural Studies*, Nos. 15, 30, 40, 47, 52, 58, 74) ; *World Health Organization : Monograph Series*, Nos. 14 (1953), 19 (1953), 33 (1957), 48 (1962).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1959, **184** ; 1967, **378** (*FAO Agricultural Studies*, 74).

hygiene [1] and meat hygiene.[2] Some of the more recent findings on specific food-borne diseases are noted in section 2 of this report.[3] Although knowledge of the epidemiology of food-borne disease is extensive, it must be continually expanded.

Problems of food-borne disease and food protection are closely related to many different environmental factors and to social and technological change. Food-borne disease and its epidemiology involve the entire chain of production, processing, and distribution of food. The level of community sanitation is important, and the role of food habits and culture is increasingly being recognized in both developed and developing countries. Investigation of food-borne disease should take all these factors into account. Rapid urbanization, technological advances, international shipment of foods, centralization of food processing, long chains of food distribution, and changing food habits have all modified the conventional approaches to the epidemiology of food-borne disease.

Changes such as those noted above have also made it necessary to modify the methods used in detecting sources of food-borne disease.

The difficulties involved in the reporting of such diseases, and the deficiencies of the methods in use, have been widely recognized. Physicians have been urged to improve their reporting to health agencies, and the latter have been requested to give greater emphasis to the detection and investigation of outbreaks. However, owing to the changing epidemiological factors noted above, a more active approach is necessary to the detection of sources of food-borne disease, and to efforts in prospective epidemiology. One such approach, which requires the co-operation of the laboratory, is the surveillance of market foods. The opportunity for accumulating information in a central agency, from which it can be readily disseminated, is one of the advantages of surveillance programmes. Adequate surveillance requires a good food hygiene laboratory that can perform tests not only for specific pathogenic micro-organisms but also for those that are indicators of hygienic quality (see section 5). Only through the accumulation of experience and information in particular local situations can informed assessments and judgements be made.

Serious attention must also be given to problems that arise in developing countries when food habits are changed through the influence of modern food technology. The inhabitants of such countries have learned to live with the risks of their environment, but new types of food (e.g., processed,

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1960, **197** (*FAO Agricultural Studies*, 52) ; Abdussalam, M. et al. (1962) *Milk hygiene*, Geneva (*World Health Organization : Monograph Series*, No. 48).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1962, **241** (*FAO Agricultural Studies,* 58) ; Albertsen, V. E. et al. (1957) *Meat hygiene*, Geneva (*World Health Organization : Monograph Series*, No. 33).

[3] The major symptoms of the principal food-borne diseases are listed in Annex 1.

precooked, and ready-to-eat foods) and differing ways of handling them introduce new risks. In such situations, food hygiene problems are likely to occur, since the necessary technology is seldom available and there may be little hygienic knowledge, food surveillance, and control.

The obligatory reporting of food-borne disease is indispensable to public health action. Reporting procedures must be simple and designed to convey as much information as possible ; however, they should not discourage the making of reports by requesting overwhelming detail. Specimen report forms and guides for the collection of samples for laboratory analysis will be found in Annexes 2 and 3.

2. RECENT FINDINGS IN FOOD MICROBIOLOGY

Some of the recent findings in food microbiology that are important for food hygiene are discussed in the following sections.

2.1 Salmonella

Salmonellosis is the most frequently reported food-borne disease of humans, and salmonellas are found as contaminants of more foods than are any other micro-organisms. Food habits and the way in which food is prepared play a major role in the occurrence of salmonellosis, but accurate epidemiological data are available for only a few countries.[1] In some areas surveillance and reporting are extremely poor, making it difficult to estimate the true situation.

Foods of animal origin, particularly red meat, poultry, eggs, and egg products, are the most important sources of salmonellosis, and animal feeds are important sources of subclinical (and sometimes clinical) infections in animals. The same is true of the Arizona group of bacteria. The following discussion refers to all salmonella types with the exception of *S. typhi* and *S. paratyphi B*, which may be spread by either water or food.

2.1.1 Human salmonellosis

The salmonellosis morbidity rate is estimated to be 10-1000 times the figures given in official reports. The disease occurs principally in those aged less than 1 year and over 60 years ; the mortality rate is also highest in these groups. The serotypes principally responsible for human morbidity are *S. typhimurium*, *S. heidelberg*, *S. enteritidis*, and *S. panama*, although in many countries the predominant serotypes vary from time to time. Of

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[1] Data for outbreaks of salmonellosis and other food-borne diseases in England and Wales during 1961-65 are given in Annex 4.

the more than 1000 different salmonella types that have been described, about 100 are observed regularly. In developed areas the salmonella carrier rate is seldom higher than 0.3 % ; however, these excretors may be a source of food contamination.

2.1.2 Salmonellosis in animals

Clinical salmonellosis occurs regularly in animals, particularly calves, horses, poultry, and piglets. However, in well-run abattoirs clinically sick animals are usually detected and eliminated by the meat inspection service, and meat from such animals therefore plays a relatively unimportant role in the transmission of salmonellosis to man. Poultry, on the other hand, do not usually undergo inspection in most countries.

In many areas the incidence of salmonella excretors in animals is extremely high as a result of certain animal husbandry practices. Animals that excrete salmonellas in their faeces, and that are not detected by the usual meat inspection techniques, are a serious hygiene problem in slaughter-houses and meat factories. Salmonella infections are readily spread among animals that are transported over long distances and held, in large numbers, in pens for fattening or slaughter. Furthermore, modern large-scale slaughter processes readily lead to cross-contamination. Salmonella infections are spread in such ways even when stringent sanitation measures are taken. On farms where salmonella excretors are present, or where clinical salmonellosis occurs, the environmental conditions may be such as to favour the spread of salmonellas over long periods of time. In addition, new contamination of the herds and flocks may occur through the introduction of new stock and from wild birds, flies, rodents and other animals, man, water, and air-borne particles. Environmental sanitation is, therefore, of the utmost importance ; however. it is extremely difficult to attain complete control of environmental contamination on farms.

S. typhimurium plays a major role in subclinical infections of animals, although many other types are found regularly. However, the types isolated from humans in a given region are not always the same as those isolated from animals. This may be the result of host-adaptation, improper isolation techniques, or other factors that are at present unknown. The host-adapted types, such as *S. pullorum* in poultry and *S. choleraesuis* in pigs, are of minor importance as causes of food-borne infections.

2.1.3 Salmonella contamination of food

2.1.3.1 *Meat and meat products*

In many countries the principal sources of human salmonellosis are pork, veal, and horse meat. Other meats, such as lamb and mutton, are also found to be contaminated from time to time, and kangaroo meat is

often contaminated. Beef is much less important, since salmonella excretors are relatively seldom found among adult cattle. Pork is often highly contaminated; large numbers of healthy excretors are found among pigs, and cross-contamination occurs readily during the complex slaughtering process. Horse meat has also been found highly contaminated with salmonellas. The hair and skin of calves are often heavily contaminated with salmonellas as a result of intensive raising procedures, and meat and organs are readily contaminated during slaughter and processing.

Salmonella-contaminated meats create a serious public health problem, particularly in countries where meat and meat products are eaten raw or insufficiently heated. For example, infections often result from the consumption of improperly cooked minced meat containing pork or horse meat. A further problem is the cross-contamination of food products from raw meat. For example, even when poultry meat is thoroughly heated, salmonellas from the raw meat often contaminate processed products, such as chicken salads and chicken sandwiches. Consequently, refrigeration and the hygienic handling of meat, at all stages of processing from the slaughterhouse to the kitchen of the consumer, are of utmost importance for the prevention of food-borne disease.

2.1.3.2 *Eggs*

Hen eggs are only rarely infected with salmonellas in the ovary; however, the shells are often contaminated and may be penetrated by salmonellas, particularly when they are cracked. Such contamination is a major problem in egg-processing plants; although it can be limited by hygienic handling, its total elimination is difficult to achieve. Several outbreaks of salmonellosis have been caused by unprocessed eggs in recent years. Recently, however, pasteurization has significantly reduced the contamination of eggs, but recontamination after pasteurization is still a problem. Duck eggs have seldom been responsible for outbreaks of salmonellosis in the past few years, apparently because the danger is so widely known by the consumer; the boiling of eggs before consumption, a procedure now widely followed, is an effective preventive measure.

2.1.3.3 *Milk and milk products*

Milk and milk products present little risk as sources of human salmonellosis, owing to the fact that pasteurization is carried out routinely in many countries and, probably, to the fact that few cows are subclinically infected. However, recontamination may occur after pasteurization— e.g., through unhygienic bottling. Occasional outbreaks of disease are caused by recontaminated products, raw milk, and powdered milk. The danger of such outbreaks is increased by the fact that milk, milk pudding, cream, and other dairy products are excellent media for the growth of

salmonellas, and special attention should be given to this problem in areas where pasteurization is uncommon.

2.1.3.4 *Fish and shellfish*

Outbreaks of salmonellosis caused by fish, oysters, and other shellfish are of major importance only in countries where they form a significant part of the diet. The risk of such outbreaks is increased if the fish or shellfish have been in contact with sewage-polluted water.

2.1.3.5 *Vegetable products*

Although there is a lack of information on the subject, raw vegetables are potentially dangerous, particularly if contaminated water or sewage is used in their cultivation. Moreover, the sprinkling of vegetables with nonpotable water, as practised in many hot countries, creates a public health hazard. In view of the increasing world trade in vegetables and vegetable products, this problem may be of greater importance than is now realized.

2.1.3.6 *Animal feed*

The feeding of animals with meal made from animal and certain vegetable material is one of the principal factors responsible for the occurrence of salmonella carriers. Such feeds are often imported (e.g., into Europe and the USA) from areas where the sanitary conditions do not meet the standards that must be maintained if the " feed–animal–food–man " cycle is to be broken. In such areas, it is difficult to avoid contamination of both raw and processed materials by birds, rodents, flies, and man. Exporting countries should, whenever possible, ensure the maintenance of hygienic standards during all stages of processing, storage, and transport so that their products will require no further treatment in importing countries. When this is not possible, the feed should be repasteurized (by steam), irradiated, or pelleted in the importing countries. It has been clearly shown that feeds treated in these ways do not cause salmonella infections in animals, whereas such infections frequently result from the use of untreated feeds.

2.1.3.7 *Cross-infection and cross-contamination*

Farms, slaughterhouses, and food-producing plants. Since relatively large numbers of animals excrete salmonellas, cross-infection and cross-contamination have become major problems, which are worsened by the holding of large numbers of animals in pens for feeding or slaughter, the transport of large numbers of animals, mass-production techniques (e.g., the slaughter of broiling chickens at rates up to 10 000 per hour), and mechanization. Contamination of processing equipment, such as work

tables, cutting machines, and utensils, may lead to contamination of large amounts of food. In recent years this problem has been encountered in many countries, particularly in the slaughter of pigs and poultry, which have to pass through scalding vats, dehairing or defeathering machines, and chilling tanks. It is also most important to prevent cross-contamination from raw to processed materials (e.g., bone, blood, and feather meal) in rendering plants.

Retail shops, kitchens, etc. The handling of food contaminated with salmonellas can easily lead to cross-contamination and create a health hazard in butchers' shops, bakeries, retail dairy shops, and similar establishments. Moreover, the mass-production methods used in canteens, cafeterias, hotels, and catering establishments increase the risk of cross-contamination. Contamination of food during its preparation has been responsible for major outbreaks of salmonellosis. Common sources of such contamination include the cutting of both raw and cooked meat on the same board ; dirty kitchen utensils, particularly those in bad repair ; dish towels ; and unwashed hands. Water dripping from thawing frozen foods, particularly poultry and meat, may contaminate the kitchen. Cross-contamination can also occur readily if raw and processed foods are stored together. If such sources of contamination are not avoided in commercial operations, large groups of consumers may be infected. On a smaller scale, the same sources of contamination are a danger in family kitchens, where housewives often are not familiar with the potential health hazards. It is important that food-handlers, kitchen personnel, and housewives be informed of the danger of salmonellosis and of the measures that should be taken to avoid contamination of food.

2.1.4 Control measures

Salmonellosis in man would become much less common if its prevalence in animals could be reduced by the use of salmonella-free feeds ; control by means of the pasteurization of all foods of animal origin is not feasible. An essential control measure that should be undertaken now is the education of food producers, dealers, and the public in the hygienic handling, cold-storage, and adequate heating of potentially contaminated products prior to consumption, and in the hazards of cross-contamination from raw to cooked products. A high standard of sanitation should be maintained on farms, during transport, and in holding pens, slaughter-houses, meat processing plants, butchers' shops, markets, restaurants, and homes.

In international trade, the systematic destruction of salmonella-contaminated food would be difficult not only for economic reasons, but also because it would cause a serious loss in the world's protein supply. However, every effort should be made to introduce progressively more stringent requirements for the freedom of food from salmonellas. In some countries

there is a tendency not to examine food for salmonellas, in order to avoid the possibility of having to condemn the food. Such a policy is to be deplored, since it does not protect the consumer.

2.2 Clostridium perfringens

Clostridium perfringens is widely distributed in soil, sewage, water, and the intestinal tract of man and animals. Several different types of the organism can be distinguished on the basis of their production of soluble antigenic toxins ; those associated with food-borne disease are type A2 and some strains of type C. Spores of *Cl. perfringens* type A are resistant to temperatures of at least 80°C for 10 min. However, it appears that most outbreaks of *Cl. perfringens* infection are caused by strains whose spores are more heat-resistant than those of typical type A strains ; furthermore, these strains have only feeble toxigenicity and little or no haemolytic effect on horse blood agar. The optimum temperature for the growth of *Cl. perfringens* is about 43°C. Its growth is restricted if curing salts, particularly nitrites, are present ; for this reason, *Cl. perfringens* infections are rarely caused by sausages and other meats prepared with such curing salts.

In epidemiological studies of outbreaks of *Cl. perfringens* infection, serological typing of strains isolated from food and the faeces of patients is valuable. Young cells that multiply actively do not readily form spores, either in cooked meat or in laboratory media, so that methods of direct culture without heating must be used for cooked foods. If large numbers of spore-forming cells are present in stools, faecal samples should be heated in broth or cooked meat media at both 100°C for 30 min and 80°C for 10 min and then incubated overnight before plating. In addition, samples should be directly plated on blood agar and incubated under anaerobic conditions.

Cl. perfringens type A may be found on the surface of meat, in muscle and lymph nodes, and in the organs (spleen, liver, and kidneys), where it is carried by the lymph and blood circulatory systems. If animals are not properly cared for immediately prior to slaughter (e.g., 24 hours' rest following transport, and feeding and watering in good time before slaughter) they are, although clinically healthy, subject to internal contamination. However, the extent of such contamination has rarely been found to be higher than 100 clostridia per gram of sample.

Since *Cl. perfringens* strains are so widely distributed, they are potential contaminants of nearly all foods, and contamination may occur in a number of ways. Raw meat and poultry are frequently contaminated before reaching the kitchen, and dust-borne contamination from vessels used for the storage of cooked food has been described. *Cl. perfringens* spores are able to survive cooking if the heat does not fully penetrate large masses of meat and poultry, which must be a common occurrence.

It is important to note that if there is a delay in the cooling of cooked meat, surviving spores will germinate, and the young vegetative cells will multiply freely in the anaerobic situations created by cooking. Furthermore, the heat of cooking will induce germination in a high percentage of spores.

Many outbreaks of Cl. perfringens infection have been traced to the eating of meat, either cold or warmed up, after it has been allowed to cool slowly in the form or large masses such as roasts, cubes, or slices. Rolled roasts are particularly dangerous, since outer surfaces, which may be contaminated, are rolled into the centre, where anaerobic conditions are good and where heat penetration and heat loss are poor.

Food-transmitted infections with Cl. perfringens would not occur if meat were eaten immediately after cooking, as is the practice in tropical and semitropical areas. The safest methods of cooking are roasting and pressure cooking, which kill spores. Roasts should not weigh more than 2–3 kg, or the heat will not penetrate them sufficiently. Meat should be refrigerated not later than 1½ hours after cooking; during this cooling period it should be placed in such a position that cool air can circulate underneath and around it. If hot sliced meat is required, it should be cut immediately after cooking and eaten either within about 1 hour or kept in preheated gravy or without gravy at a temperature greater than 60°C until eaten. Rolled roasts should always be well cooked, and preferably eaten immediately.

2.3 Bacillus cereus

Although Bacillus cereus may be a cause of food-transmitted disease, the illness is short and rarely if ever has a fatal outcome ; it has been reported principally from Norway, Sweden, and Hungary. The symptoms resemble those caused by infection with Clostridium perfringens ; outbreaks of the two infections also show certain epidemiological similarities. Since B. cereus spores can survive heating, vegetative forms of the organism may multiply rapidly in a suitable precooked food that is left to cool slowly.

B. cereus occurs in soil and dust and on plants. Potatoes and other vegetables often carry the organism, and from them it may contaminate other foods. B. cereus is also found in large numbers in sausages, milk, and egg powder, and it is a common contaminant of corn flour (corn starch) and potato starch, which are ingredients of vanilla-sauce (custard) powders. Vanilla sauce and similar foods have frequently caused illness when prepared a day in advance and stored under conditions permitting the growth of B. cereus. (The syndrome has been reproduced by the consumption of inoculated vanilla sauce in which the organism has been allowed to grow ; however, the consumption of filtrates from cultures has given negative results.)

The best way of preventing *B. cereus* infection is to avoid long incubation periods before food is served. If long storage after cooking cannot be avoided, the food should be rapidly cooled to a temperature below 10°C to retard the multiplication of the bacilli.

Since it is not possible to reduce the prevalence of *B. cereus* or *Clostridium perfringens* in nature, it must be accepted that both organisms will be present in food, although the incidence of such contamination and the number of organisms may be reduced by the maintenance of good hygiene. Neither organism is dangerous when ingested in small numbers. Consequently, the primary aim of preventive measures should not be their destruction, but the control of spore germination and subsequent multiplication of vegetative cells in heat-treated foods.

2.4 Clostridium botulinum

Human botulism results from the consumption of food in which *Clostridium botulinum* has grown and produced its toxin. Six immunologically distinct types of the organism are known. Most of the recognized outbreaks of botulism in man have been caused by types A, B, and E ; types C and D are usually associated with the disease in animals, such as mink, waterfowl and cattle and other domesticated species ; type F has been reported only rarely.[1]

Botulism occurs only rarely in humans, since the conditions necessary for an outbreak seldom exist. Except in a few special instances, botulinum poisoning occurs only if all of the following requirements are met :

(1) The food must be contaminated with *Cl. botulinum* spores. Since the organism is so widely distributed in nature, its presence on raw food may be assumed.

(2) The food must be subjected to some treatment that will destroy (or inhibit the growth of) the normal contaminating microflora while allowing spore formers to survive. These conditions will be brought about by procedures such as mild heating, salting, and pickling. This requirement need not be met in certain outbreaks caused by the following foods : Japanese " Izushi ", which is prepared by allowing a mixture of raw fish, rice and vegetables to ferment at room temperature, and " fermented " fish eggs and the partly decomposed flesh of whales and other mammals, which are sometimes eaten by native tribes in the northern hemisphere. In outbreaks traceable to such foods, the causal agent is usually the nonproteolytic *Cl. botulinum* type E.

(3) The composition of the food must be suitable for the growth of *Cl. botulinum* and for toxin-formation by the multiplying organism. In

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[1] Methods for the identification of the different types of *Cl. botulinum* are briefly discussed in Annex 5.

general, growth can occur if the pH is above 5.0 and the equilibrium relative humidity is above 94%.

(4) The food must be held at a suitable temperature for sufficient time to allow growth and toxin formation. *Cl. botulinum* type A and B can grow at temperatures above 10°C and types E and F at temperatures above 3°C.

(5) The food must not be cooked before it is eaten. Botulinum toxin is relatively sensitive to heat ; exposure to a temperature of 80°C for 30 min or to boiling for a few minutes is sufficient to inactivate the toxin.

The requirements listed above are met by the foods most commonly involved in outbreaks of botulism—e.g., underprocessed home-bottled vegetables ; lightly salted cured meats ; fish pickled without sufficient acid ; and lightly salted, smoked, and partly dried fish.

Botulism can be prevented by any of the following measures :

(1) destruction of the spores by heating or irradiation ;

(2) inhibition of growth by (*a*) reduction of the pH (through acidification or fermentation), (*b*) limitation of the water content (through drying or the addition of salt or sugar), (*c*) reduction of the temperature (through freezing or refrigeration), or (*d*) addition of inhibitory chemicals such as nitrites ; and

(3) inactivation of preformed toxin by cooking.

Though relatively labile to heat, botulinum toxin is highly stable under acid conditions and will persist for long periods in foods with a pH below 6.0. In laboratory work with botulinum toxins, the pH should be kept at 6.0 or less to avoid inactivation.

The high world-wide mortality rate (over 50% of the reported cases) in outbreaks of botulism has stimulated research wherever the disease has been recognized. In recent years the emphasis in such research has been given to *Cl. botulinum* type E, which is usually associated with fish and other animal products from the aquatic environment. Once thought to occur only in localized areas of the seas north of the 40th parallel, this organism is now known to exist in both fresh and salt water in many parts of the world. Particularly high concentrations have been found in the Baltic Sea, the coastal waters of Hokkaido, Japan, and parts of the North American Great Lakes. The concentration of the organism varies widely, even in adjacent areas ; furthermore some areas appear to be free of type E spores. The causes of localized heavy concentrations of type E spores are not known ; the organism occurs sparsely in soil, but in certain bodies of water the conditions are apparently suitable for multiplication.

The association of botulism outbreaks with vacuum-packaged smoked fish in the USA has raised questions about the possible hazard of the

vacuum packaging process. Moreover, there is confusion over the suscep-
tibility of smoked fish to botulinogenesis. Several laboratories have clearly
demonstrated that vacuum packaging has no significant effect on the final
production of toxin by *Cl. botulinum* in a food product, although toxin
production may initially be favoured by reduced oxygen partial pressure.
In whatever way it is packed, a mass of meat, fish, or other suitable food,
whether sliced or solid, provides suitable conditions for the growth of
Cl. botulinum, if the organism is present. Vacuum packaging may contribute
to the botulinum hazard only through its inhibition of moulds and other
aerobic micro-organisms, thereby permitting longer storage and misuse
of the product. If the product is one that supports the growth of *Cl. botu-
linum*, the toxin may be formed without signs of mould growth or other
visible evidence.

The smoking of fish is essentially a flavouring process. Many types
of smoked fish are produced ; some contain insufficient water for the growth
of *Cl. botulinum*, whereas others are quite moist and may serve as an
excellent medium for toxin formation, unless adequately salted. Thus, in
estimating the hazard of smoked fish, the composition of the product and
its suitability for the growth of *Cl. botulinum* must be considered.

Cl. botulinum type E differs from the better-known types A and B in
the following important characteristics.

(1) In an aqueous environment (equilibrium relative humidity near
100%), type E spores are killed at 80°C within a few minutes. Spores of
types A and B, on the other hand, may survive for hours at temperatures
above 100°C. The resistance of type E spores is greatly increased in the
dry or semidry environment that may exist in smoke chambers and similar
heating devices.

(2) Since *Cl. botulinum* type E will grow at temperatures as low as
3°-4°C, even properly refrigerated foods constitute a hazard. Types A
and B do not grow below 10°C.

(3) The limiting salt concentration for the growth of type E is approx-
imately 5% and that for types A and B is 8–9%, the percentages being
based on the concentration of sodium chloride in the aqueous phase of the
food or culture medium.

(4) The toxin titre of type E cultures is relatively low (1000–2000 mouse
lethal doses per ml of culture), but it increases by a factor of 100–300 on
treatment with certain proteolytic enzymes, such as trypsin. The same is
true of certain nonproteolytic strains of type B. Proteolytic *Cl. botulinum*
cultures (type A and most type B strains) show relatively high initial toxicity,
with no activation or increase by trypsin.

Specific treatment for botulism is limited to antiserum therapy. A
dramatic reduction of the mortality rate from type E botulism has been
achieved in Japan by the administration of type E antitoxin as soon as

the disease is diagnosed. The effectiveness of types A and B antisera has not been adequately evaluated, owing to the frequency of late diagnosis. If, in an outbreak of botulism, the specific type is not known, polyvalent antisera containing a mixture of type A, B, and E antitoxins may be administered. Laboratory personnel working with *Cl. botulinum* cultures, and other individuals with high risk, can be actively immunized with toxoid ; a mixed toxoid for types A, B, and E is often used.

2.5 Staphylococcus

The multiplication of certain strains of staphylococcus in foods leads to the appearance of enterotoxin. It now seems certain that enterotoxin is produced solely by the so-called " enterotoxic " strains. In general, these strains show the usual characteristics of pathogenic staphylococci and in addition secrete coagulase. However, food-borne coagulase-negative strains have also been reported to cause intoxication.

Enterotoxin can now be isolated and purified,[1] and three antigenic types have been described and designated A, B, and C. They are identified by the gel-diffusion technique, in the presence of homologous antitoxic sera ; however, the techniques for extraction, purification, and identification are still difficult and can be used only in highly specialized laboratories. For routine work, simpler and more specific identification methods are necessary. Phage-typing is useful for identifying causative organisms, and may give a clue to the origin of contamination in food, since certain phage types are found more frequently than others in enterotoxin food poisoning.

Contamination of food products may be present originally or be of indirect origin. Contamination of the former type arises when food is derived from a sick animal, the commonest source of infection being bovine mastitis. Indirect contamination is usually derived from a food handler who carries the bacteria on the skin of his hands or forearms (in conditions such as pyodermitis, infected wounds, and furunculosis) or in his rhinopharynx. The staff of processing plants should be watched for clinical signs of such conditions, and persons showing them should be prevented from coming into contact with food.

To be pathogenic for man, enterotoxin must be present in a foodstuff in sufficient quantity ; substantial multiplication of the contaminating staphylococci is, therefore, necessary. Conditions favourable to such multiplication include the following :

(1) A relatively high temperature. Multiplication occurs only slowly at 10°C, but increases progressively with temperature, reaching a maximum at 30–40°C.

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[1] For notes on methods, see Annex 6.

(2) A fairly high pH. No appreciable multiplication occurs in acidic products (i.e., those having a pH of 4.5 or less).

(3) The presence of the substances (proteins and carbohydrates) that can be most rapidly utilized by the organism, or that are indispensable to its growth.

In common with other Micrococcaceae, staphylococci tolerate a low water activity (0.85)[1]. They can, therefore, multiply in products with a relatively high salt or sugar content.

In contrast to botulinus toxin, which is thermolabile, staphylococcal enterotoxin that is preformed in foodstuffs is thermostable, and has been shown to withstand boiling and even higher temperatures. This explains the relatively more frequent occurrence of enterotoxin in foodstuffs such as the following, which have been involved in the transmission of disease : meat and meat products (e.g., meat pies, corned beef, and ham); custards and similar products; cheese; sweetened, condensed, and powdered milk; and certain canned foods, particularly sardines.

The most effective means of prevention are as follows :

(1) rapid refrigeration of food products, to prevent the multiplication of contaminating staphylococci;

(2) control of human carriers in critical points in processing plants, distribution circuits, and kitchens; and

(3) maintenance of hygienic standards in processing plants, during transport, and by distributors, retailers, and consumers.

2.6 Vibrio parahaemolyticus

Vibrio parahaemolyticus food poisoning has so far been reported only from Japan, where it was first observed in 1953. This may be a result of the Japanese preference for raw fish; however, the organisms have been shown to be present in France, South-East Asia, and the states of Washington and Hawaii in the USA, particularly in the mud of seashores and estuaries. In Japan, a number of outbreaks of food poisoning, mostly in the summer, have been caused by the consumption of salt-water fish. Shellfish caught in estuaries have also caused outbreaks, and various fish products have been blamed. Many of the outbreaks have resulted from the eating of freshly-caught fish; *V. parahaemolyticus* has been found to grow with unusual rapidity in culture media and in fish and other foods. In cases of *V. parahaemolyticus* food poisoning, the incubation period is usually 15–17 hours, and the chief symptoms are abdominal pain, watery diarrhoea, and vomiting.

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[1] Water activity, a way of expressing the available moisture content, is the vapour pressure of the solution divided by the vapour pressure of water.

It has been reported that the organism produces substances toxic to mice, but the role of toxins in the causation of disease has not been clarified. For this reason, *V. parahaemolyticus* food poisoning should, for the time being, be classified as an intestinal infection caused by a large number of the living organisms in food.

The diagnosis of the illness is entirely dependent upon isolation of the organisms from the faeces or vomitus of patients and from foodstuffs, and upon biochemical and serological identification of the isolated organisms (see Annex 7).

2.7 Mycobacterium avium

It has been generally believed that human beings are not susceptible to tuberculous infection caused by *Mycobacterium avium*. However, a recent review [1] has shown that 97 cases of tuberculosis have been reported as having been microbiologically proved to be the result of *M. avium* infection.

Epidemiological studies have shown that there are substantial differences in the ways in which avian mycobacterial infections and typical human tuberculosis are transmitted to man. *M. avium* appears to be transmitted by infected chickens, eggs, raw milk, pork, and beef.

Future reports of human infections with avian tuberculosis may be expected from regions where the disease persists in poultry and other animals, although effective control of human and/or bovine tuberculosis may have been achieved.

Further studies are necessary of the sources of *M. avium* contamination in food and of the persistence of the organism in foods, including its apparently high resistance to food-processing procedures.

2.8 Viruses and Rickettsias

Until recently, there has been a lack of reliable techniques for the laboratory study of many of the viruses, and there has been little investigation of the role of food in the transmission of viral diseases.

However, it is known that food products, particularly meat, milk, seafood, and vegetables, act as vehicles for the transmission of viral and rickettsial diseases to man. Such foods may be originally infected, or they may become contaminated by food handlers or the environment. It has been shown experimentally that some enteroviruses (coxsackieviruses, poliovirus and echoviruses) can be transmitted by water and uncooked vegetables. Tick-borne encephalitis virus (an arbovirus) and the Q-fever organism (a rickettsia, *Coxiella burnetii*) have been shown to be excreted in the milk of sheep, goats, and cattle. Many persons have been infected with tick-borne encephalitis through the consumption of unheated products

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[1] Kubin, M. et al. (1966). *Amer. Rev. resp. Dis.*, **94**, 20.

made from goat's milk. There is conclusive epidemiological evidence of the transmission of the infectious hepatitis virus to man through oysters, clams, and other foods that are consumed unheated.

Foot-and-mouth disease virus very rarely causes human infection ; it is not clear whether contact with infected tissue or the consumption of unheated milk is the primary route of transmission. Man can acquire infection with Newcastle disease virus by contact with infected poultry ; this virus is eliminated in eggs, but it is not known whether transmission occurs by the oral route.

Many viruses can infect both animals and man. The strains that infect man and those that infect animals are usually not identical, but are closely related in certain characteristics. Much additional work is required to determine the importance and infectivity of such viruses for man, as well as their possible presence in food products. These viruses include avian and swine influenza A viruses ; rhinoviruses of cattle, which cause the common cold in man ; reoviruses, parainfluenza viruses, and herpesviruses of cattle and other animals ; and the avian leucosis virus. Little is known of the heat resistance of most of these disease agents, and much of the available information has been obtained under experimental conditions that are of little relevance to food hygiene. Many viruses are more resistant to heat than are vegetative bacteria, which are usually killed by pasteurization processes now in use. It has been reported that *Coxiella burnetii* and some coxsackievirus strains can survive certain vat-holding pasteurization procedures. Chlorination of water appears to have less effect on viruses than on bacteria.

Because of the specialized nature of the techniques that are required, food-hygiene laboratories do not routinely examine food for the presence of viruses. Further study of problems arising from the presence of viruses and rickettsias in food is urgently needed. In particular, it is recommended that attention be given to the simplification of laboratory procedures for the detection of these organisms, and to their resistance to heat and other food-processing procedures.

2.9 Mycotoxins

Literally hundreds of toxic compounds produced by actinomycetes and other fungi are known ; one of the first to be recognized was that contained in the sclerotia of the ergot fungus *Claviceps purpurea*. A review of the mycotoxin problem has recently been published.[1] Interest in the subject increased in 1960, when more than 100 000 young turkeys died in England.[2] Since the discovery that aflatoxin, a metabolite of *Aspergillus flavus*, causes

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[1] US National Academy of Sciences (1966) *Toxins occurring naturally in foods*, Washington, D.C.

[2] Sargeant, J. J. et al. (1961) *Vet. Rec.*, 73, 1219.

a liver disease in turkeys, ducklings, pigs, and calves, this compound has been studied extensively.[1] First found in peanuts, aflatoxin has also been reported to occur in maize, rice, sorghum, soya beans, wheat, cotton, and cotton seed meal. Both qualitative and quantitative errors may readily occur in the estimation of aflatoxin by the fluorescence-emission methods now in use, and confirmatory tests are essential to ensure correct results. The International Union of Pure and Applied Chemistry is now carrying out an international study of methods for the determination of aflatoxin, with a view to developing a standard test.

The Codex Committee on Food Additives has been requested, when determining matters for future toxicological evaluation by the Joint FAO/ WHO Expert Committee on Food Additives, to give high priority to toxic substances that occur naturally in food—e.g., aflatoxin and gossypol.

Most of the toxin-producing fungi are world-wide in distribution, so that it is impossible to restrict them to certain areas as has been possible with many other organisms that cause disease in plants and animals. For example, *Aspergillus flavus* has been reported from all except the coldest parts of the world, where it will not grow. Although toxins are formed at various temperatures, certain toxins are produced only at low temperatures ; for example, it has been shown that *Aspergillus flavus* grown at 37°C produces little or no aflatoxin even though high-aflatoxin-producing strains are used on a suitable substrate. Under appropriate conditions, fungi can attack every known plant and animal material ; thus, every agricultural commodity is open to attack, and every damaged commodity represents a potential source of mycotoxins.

The most effective and practical method of preventing the growth of moulds is thorough drying. The method of drying seems to be of great importance ; for example, it has been found that slow drying of groundnuts is undesirable and excessively rapid drying unsuccessful.[1] Other means of preventing the growth of fungi, such as the use of fungicides and anti-microbial agents, should be studied.

Finally, the Committee endorses the statement on mycotoxins in food made in the seventh report of the Joint FAO/WHO Expert Committee on Nutrition (see Annex 8).

2.10 Animal Parasites

Although metozoan animal parasites are not usually covered in food microbiology, they cause important food-borne infections. Since the infective stages of such parasites that occur in food are usually microscopic in size, the Committee considers it necessary to draw attention to their

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[1] For a review of recent work, see *Food cosmet. Toxicol.*, 1967, **5**, 404.

importance in food hygiene, without going into details of their epidemiology and control [1] except to note factors of importance to food processing.

From the point of view of food hygiene, the food-borne animal parasites can be conveniently grouped in two categories :

(1) Parasites that are present in the tissues of food animals, where they may or may not undergo cyclic development, and that persist in the food —e.g., meat, fish, and shellfish—in a form that is infective to man (*Taenia saginata*, *T. solium*, *Diphyllobothrium latum*, *Clonorchis* and *Opisthorchis* spp., *Paragonimus westermani*, *Trichinella spiralis*, *Anasakis* spp., *Angiostrongylus cantonensis*, and possibly *Toxoplasma*). Transmission depends on the consumption of raw or insufficiently processed food.

(2) Parasites that are derived from the environment (soil or water), from animals, or from food handlers and whose infective stages are transmitted in food (*Ascaris lumbricoides*, *Toxocara canis*, *Echinococcus* spp., *Fasciola* spp., *Fasciolopsis buski*, *Entamoeba*, and *Giardia*). Transmission depends on the consumption of insufficiently processed food or food that has been recontaminated after processing or preparation.

Certain animal parasites (e.g., nematodes and *Sarcocystis*) may be present in meat and fish in such large numbers as to render the food unwholesome, even though their transmission to man has not been established.

Control

The best method for the control of infections with food-borne animal parasites is the elimination of such parasites from food animals and from the environment.[1] Until this is attained, control must depend on the processing of infected food during its production or preparation and on the prevention of recontamination. Such processing should not seriously affect the quality and acceptability of the food. A further important control measure is health education of the public, who should be informed of the danger of eating raw and under-processed food that is likely to be infected.

3. FOOD TECHNOLOGY IN RELATION TO FOOD HYGIENE

The usual purposes of food processing are (1) to change the form or characteristics of the product so as to make it easier to market and more attractive to the consumer ; (2) to inhibit or eliminate factors that might cause deterioration or spoilage ; (3) to kill any pathogenic micro-organisms that might be present ; and (4) to improve the nutritive value of the food.

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[1] For further information, see *Wld Hlth Org. techn. Rep. Ser.*, 1962, **241** ; 1967, **378** (*FAO Agricultural Studies*, 58 and 74).

Whatever the purpose of processing, it may introduce or fail to eliminate certain types of microbial flora ; thus, all food processing has important implications for hygiene. Furthermore, the care of products after processing also has implications for food hygiene ; special care may be necessary to achieve the purposes of processing or to make it possible to market and use a given product.

3.1 Thermal Processing

3.1.1 Indefinitely stable products

A stable food is one that is processed in such a way as to reduce the number and/or activity of viable micro-organisms to such an extent that very few, if any, are detectable in the treated food by any recognized method. No spoilage or toxicity of microbial origin is detectable no matter how long or under what conditions the food is stored, provided it is not recontaminated.

Food products having a pH above 4.5 must be processed in such a way as to destroy spores of *Clostridium botulinum*. These products include canned meats, canned vegetables, and other products of high pH that are packed in hermetically sealed containers. Such products are indefinitely stable, unless recontamination occurs as a result of imperfect sealing of the container. However, cold-storage is desirable to prevent deterioration of the quality of the product.

Food products having a pH below 4.5 require less thorough processing, since organisms such as *Cl. botulinum* cannot germinate and grow in a strongly acidic environment. Such products include acid fruits, acid vegetables, and pickles in hermetically sealed containers. To make such products indefinitely stable, they need only be processed in such a way as to prevent the growth of organisms that might cause spoilage.

Provided that indefinitely stable processed food products are not exposed to contamination, they will cause no microbiological problems.

3.1.2 Products of limited stability

The texture and flavour of certain products are affected by processing that destroys spores of *Cl. botulinum*. For example, hams are often marketed in hermetically sealed cans ; such hams are subjected to a mild heat treatment that is sometimes referred to as pasteurization. Since such products are not indefinitely stable at room or higher temperatures, they must be kept under refrigeration. Occasionally, such products of limited stability contain toxigenic staphylococci and, if not properly handled, may present a health hazard. To protect the public against food poisoning, the marketing of such products should be subject to strict control.

3.1.3 Perishable products

3.1.3.1 *Pasteurized products*

Milk and fruit juice are examples of perishable products that are subjected to pasteurization. Milk usually contains a wide variety of micro-organisms, many of which can cause spoilage, and unless pasteurized and refrigerated it will quickly be spoilt. Pasteurization reduces the number of micro-organisms to such an extent that, when properly packaged, handled, and refrigerated, the product will remain in satisfactory microbiological condition for a longer time.

In addition to micro-organisms that cause spoilage, milk may contain pathogens, and this possibility must be taken into account in establishing the temperatures and other conditions to be used in the pasteurization process.[1] Milk products must be properly handled and protected from recontamination after pasteurization.

Fruit juices present far fewer problems than milk, since their pH is usually low and spoilage usually results only from the growth of yeasts and fungi ; there is rarely any problem caused by the growth of pathogens or toxin producers. Consequently, a much lower pasteurization temperature may be used than is necessary for products such as milk ; treatment that destroys the yeasts is usually sufficient. Most pasteurized fruit juices are microbiologically stable at room temperatures. Certain fruit juices that are produced for local consumption may be pasteurized only to a degree that permits storage of reasonable duration under refrigeration.

3.1.3.2 *Precooked products*

Certain precooked products that contain several ingredients are difficult to pasteurize ; each of the ingredients may contaminate the mixture with a different group of organisms. In treating such products to control spoilage, it must be borne in mind that salmonellas may be present, and they must be heated sufficiently to ensure the killing of pathogens. However, such treatment does not destroy spore-forming micro-organisms that may be present in vegetable and cereal ingredients, and the products must, therefore, be refrigerated or frozen. Recontamination of such food products is particularly dangerous, since they usually have a high pH and provide almost perfect nutritive conditions for micro-organisms, which will grow rapidly if the products are allowed to stand at elevated temperatures.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1957, **124** ; 1960, **197** (*FAO Agricultural Studies*, 30 and 52) ; Abdussalam, M. et al. (1962) *Milk hygiene*, Geneva (*World Health Organization : Monograph Series*, No. 48).

There is such a wide variety of food products of this type that it is not feasible to discuss them all. Furthermore, since their pH may vary widely and since the contaminating organisms that may be present will depend upon their ingredients, no single set of temperatures or other conditions can be established for the pasteurization of such products. It can only be pointed out that pasteurization must be adequate to eliminate potential health hazards. It is particularly important that the products be properly packaged and protected from recontamination.

3.2 Freezing

A wide variety of frozen foods, including meat, poultry, fish, shellfish, fruit, vegetables, and mixtures of these products is marketed in large quantities in many countries. Since many such foods are frozen raw, the control of microbial flora by hygienic handling is all that can be relied upon to render them safe. The freezing process usually tends to protect micro-organisms, and a reduction in microbial content can be expected only after long storage. However, even long storage does not ensure the elimination of pathogens.

Meat, poultry, and fish usually contain many different micro-organisms, the number and type of which can be used to estimate the level of hygiene maintained in the preparation of the products for freezing. Freezing permits the marketing of food products over a wide geographical area and at different times of the year. Since they are so widely distributed, it is essential that frozen foods present no health hazard ; this can be achieved only by hygienic preparation, handling and storage and by the prevention of recontamination by adequate packaging.

When frozen foods are removed from the freezer, bacterial action will begin as soon as the temperature rises. The refreezing of thawed foods should be avoided.

Frozen mixed foods may be either raw, in which case they are cooked before consumption, or precooked, in which case they are only warmed up before consumption. It is most important that such products be protected by adequate packaging against contamination and that they be cooked sufficiently to ensure that they present no health hazard. Special products such as frozen dairy products, sherbets, sauces, and ready-to-bake fruit and meat pies must be hygienically prepared and adequately packaged.

3.3 Dehydration

Many types of dehydrated food are marketed, and each presents a particular microbiological problem. Dehydrated products are advantageous to the food industry in that their weight is markedly reduced and their

keeping quality is good. Chemical additives are not usually used in such products.

3.3.1 Spray drying

In spray drying, liquid products such as skimmed milk and eggs are sprayed into a rapidly moving current of hot air that removes the moisture and leaves a powder of low moisture content. Such products should have a low viable microbial content before they enter the drying chamber ; for this reason, they are usually pasteurized before being dried. It is important that the temperature of pasteurization be high enough to kill any pathogens that may be present. The drying operation cannot be depended upon for the control of pathogens since, although the temperature in the drying chamber is high, the evaporation of moisture cools the small food particles so that they seldom reach a temperature high enough to kill micro-organisms.

Spray-dried foods are quite hygroscopic and require protective packaging. This is a marked advantage from a microbiological point of view, since it prevents recontamination ; furthermore, the maintenance of a low moisture content helps to prevent the multiplication of moulds and other micro-organisms. If such products are found to contain pathogens, such as salmonellas, a check should be made to discover the fault in the processing.

3.3.2 Hot-air drying

Fruits, vegetables, and some meats may be dried on trays in hot air tunnels. In such driers, micro-organisms in foods undergo multiplication only if the trays are overloaded or if the food is improperly distributed ; if there is free and adequate circulation of hot air, such growth will not occur. Although drying markedly reduces microbial populations, it cannot be depended upon to ensure a hygienic product. The raw foods must be prepared and handled in such a way that they are safe and sanitary when they enter the drier. To prevent changes in colour and flavour, many fruits are treated with sulfur dioxide which, together with the naturally low pH of most fruits, gives some protection against microbial contamination. However, vegetables and meats whose pH is high have no inherent protection, and contamination must be rigidly avoided.

Hot-air-dried foods are, like spray-dried foods, hygroscopic and must be protectively packaged.

3.3.3 Open-air drying

In some countries fruit, fish, and certain meat products may be dried in the open air. Products dried in this way are exposed to air-borne con-

tamination and to contamination by insects and birds. Little can be done to control the microbiological content of such foods, and the only safeguard is adequate cooking by the consumer. Although open-air drying is undesirable, foods dried in this way are consumed in large amounts.

3.3.4 Freeze-drying

Freeze-drying preserves the natural texture and appearance of foods to a much greater extent than does conventional hot-air drying. In the freeze-drying process, foods are solidly frozen, usually in the form of pieces or fairly thin sheets, placed in a vacuum dehydrator, and subjected to a high vacuum. Moisture evaporates from the surface of the frozen product by sublimation—i.e., it passes from the solid state to the vapour state without liquefying. Thus, the original structure and dimensions of the product are maintained. After completion of the process, the product is a spongy mass whose water content is only 2–5%. A further advantage of freeze-dried products is the fact that they can be rehydrated rapidly.

Meat, poultry, fish, shellfish, vegetables, fruit and a wide variety of other foods are freeze-dried, either raw or cooked. Since freeze-drying kills few bacteria, products prepared in this way contain essentially the same microbial flora as was present before freezing; in fact, the process has been used to preserve bacterial cultures. For this reason, good sanitation is essential in the preparation of foods for freeze-drying, and microbiological sampling of most raw products is advisable. If such sampling shows the presence of large numbers of viable micro-organisms, or if pathogens are found to be present, the hygiene being practised in the preparation of the raw materials should be checked immediately. Freeze-drying entails considerable handling of the product by workers and strict sanitary standards must be maintained.

Freeze-dried products are stable at room temperature but, if to be stored for a long time, will retain better quality if refrigerated. As with spray-dried and hot-air-dried foods, protective packaging is necessary.

3.4 Use of Microbe Inhibitors

A wide variety of foods are preserved by means of substances that retard or inhibit the growth of certain micro-organisms. However, since the effect of such inhibitors is bacteriostatic rather than bactericidal, living micro-organisms remain on the food products.

3.4.1 Natural inhibitors

Certain vegetable products are prepared in such a way as to bring about a natural fermentation that produces inhibitors. For example, in

the preparation of sauerkraut, the fermentation of shredded cabbage produces enough lactic acid to inhibit the growth of organisms that might spoil the product, which will remain in good condition for several months provided oxygen is excluded.

Certain cucumber products that are allowed to ferment in brine will, if sealed from the air, remain in good condition for long periods owing to the preservative action of the salt and the naturally formed lactic acid. However, cucumbers that are fermented in low-salt brine will not keep for as long a time. Cases of food poisoning have not, as far as is known, been caused by such cucumber products.

Salted herrings that undergo lactic-acid fermentation in barrels will remain in good condition for long periods of time in sealed containers. Following the addition of an acidic sauce, the herrings may be repacked in tin cans and sealed without heat treatment. Only rarely have cases of food poisoning from this type of product been reported, and those cases probably resulted from faulty processing (e.g., an improperly sealed can).

Many foods can be satisfactorily preserved by the use of acetic acid or vinegar, an acid fermentation product; the food itself does not undergo fermentation. The food will keep better if the vinegar is heated and the package sealed while hot; such vacuum packing inhibits the growth of aerobic bacteria that might cause spoilage. Acetic acid is sometimes used in the preservation of certain meat and fish products, usually together with a mild heat treatment.

Many types of cheese undergo a type of fermentation, brought about through the use of natural or " starter " cultures, that contributes to their preservation. The free fatty acids and other compounds that are formed by this fermentation enhance the keeping quality of the cheese and produce its characteristic flavour and aroma. Food-poisoning organisms and other pathogens that enter the cheese after it is pasteurized can survive all the remaining processing steps, and may cause serious health hazards. One case of botulism was traced to a particular type of cheese. These facts point to the necessity for constant surveillance of cheese making to ensure a hygienic product.

In alcohol fermentation, sugar is converted into alcohol; thus, one of the nutrients necessary for yeast growth is removed and at the same time the alcohol content is raised to a level that inhibits the growth of organisms that might cause spoilage. However, products that are processed by alcohol fermentation must be sealed to exclude oxygen. Such products do not normally undergo spoilage, and they have very rarely given rise to microbial health hazards. Certain products may also be preserved by the addition of alcohol; for example, if the alcohol content of wine is increased to about 21% by the addition of brandy, spoilage will be prevented even though the wine may be sweet.

3.4.2 Salt

Many products are preserved with dry salt or with brine. Dry-salted meat and fish can be kept without deterioration for long periods of time, since the high salt content of the tissues inhibits both bacterial and enzymatic action. Nitrates and nitrites may be added to brines ; these salts not only help to preserve the product but protect its colour from fading. Salt-tolerant organisms occasionally grow in brines, causing them to become " ropy " or the product to become sour. Pathogens may persist in brine and brine-cured products.

3.4.3 Smoke

Certain meat products, such as ham and bacon, are smoked after being cured in salt. The smoke dries the surface of the meat and contains substances that retard or inhibit the growth of micro-organisms. A similar treatment is sometimes used to preserve turkey, chicken, and other types of meat.

Some types of sausage contain curing ingredients and undergo lactic-acid fermentation ; they are then placed in a smoke house and subjected to mild heat. This treatment produces a characteristic flavour and excellent keeping qualities.

Many kinds of fish (e.g., salmon, sturgeon, chub, and eel) are subjected to a smoking process that slightly cooks them, dries their surface, and deposits on them substances that act as surface preservatives. Such products are usually not salted, or are salted very lightly, and have occasionally given rise to cases of food poisoning. On rare occasions, fatal cases of poisoning with *Clostridium botulinum* type E have resulted from the consumption of smoked fish. It should be noted that smoking, curing, and brine-preserving processes do not guarantee the absence of pathogens.

3.4.4 Chemical inhibitors

Chemical inhibitors such as sodium benzoate, sorbic acid, trichloro-acetic acid, and sodium propionate are used in certain products. Such substances are usually used to control specific types of spoilage-causing organisms, and are not effective against a wide variety of micro-organisms.

3.4.5 Antibiotics

Some countries permit the use of antibiotics [1] to inhibit the growth of spoilage-causing organisms in certain foods.

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[1] For further information, see : *Wld Hlth Org. techn. Rep. Ser.*, 1963, 260.

3.5 Control of Water Content by Addition of Sugar

Microbial growth can also be controlled by minimizing the water content of foods. This method has been covered to some extent in other sections (e.g., section 3.4.2).

Sugar acts as a preservative when in high concentrations. Concentrated sugar syrups do not undergo spoilage, since their low water activity [1] inhibits the germination of spores and the growth of vegetative cells. Honey does not spoil for the same reason, although its sugar concentration is occasionally insufficient to inhibit the growth of certain osmophylic yeasts, and fermentation will take place.

Fruits can be preserved in concentrated sugar solutions or by crystallized sugar (e.g., glacé fruits). Sugar is sometimes used in the curing of meat, partly to enhance the flavour.

The available water content of sweetened condensed milk (produced by pasteurization, concentration, and the addition of sugar) is sufficiently low to preserve the product. The presence of pathogens or the occurrence of spoilage in such a product is an indication that contamination or leakage of the container has occurred, and indicates the necessity of surveillance of the raw materials and of the procedures used in processing.

3.6 Control of Microbial Flora in Raw Foods

3.6.1 Meat

Meat rapidly undergoes spoilage, since it is an excellent source of nutrients and both spoilage-causing bacteria and pathogens grow rapidly in it at room temperature. Since meat is used in the raw state by consumers, it is necessary to prevent spoilage long enough for it to be marketed in this state. The most widely used method of preservation is refrigeration. As the temperature approaches 0°C, the growth of spoilage-causing organisms is markedly retarded. Below 3°C and at an appropriate humidity, meat retains its freshness for a relatively long time. However, certain cold-tolerant organisms grow slowly at refrigeration temperatures, producing a slimy surface on, and eventually spoiling, meat products. Relatively few pathogens will grow on meat below 10°C; thus, to ensure the prevention of health hazards, the temperature at which fresh meat is stored must be carefully controlled.

The contamination of fresh meat prior to refrigeration must be kept to a minimum by hygienic production and handling, to ensure a low microbial population and the absence of pathogens. The meat should then be chilled and placed in cold storage as rapidly as possible, so that

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[1] See footnote 1, p. 19.

micro-organisms that may have accidentally contaminated it will not grow and cause a health hazard.

3.6.2 Fish

Fish presents essentially the same problems as meat, except for the types of contamination involved and the fact that fish has no animal heat and is easier to chill. Fish do not naturally carry a wide variety of pathogens, and those that may contaminate the marketed product are usually derived from the environment—e.g., pollution of the water in which they live and improper handling after they are removed from the water. In order to produce fish of low pathogen content, it is essential that it be taken from a sanitary environment and kept chilled during all stages of processing and marketing. Although fish carry *Clostridium botulinum* spores, no cases of botulism have resulted from the eating of fresh raw fish. However, cases of *Vibrio parahaemolyticus* poisoning have been caused by the consumption of such fish. Salmonellosis does not usually result from the consumption of properly handled fresh fish, but cases have been caused by improperly handled fish products. Shellfish caught in contaminated waters are an important source of infectious hepatitis and other food-borne disease.

3.6.3 Vegetables

The surfaces of fresh vegetables are likely to be contaminated with a wide variety of micro-organisms, depending upon the agricultural practices used in raising them. Pathogens of animal origin will occur on vegetables raised with the aid of manure or polluted water. Pathogens from man are particularly likely to be present on vegetables raised in countries where night soil is used as a fertilizer or where sewage-contaminated water is used for irrigation. Much of the contamination can be removed by thorough washing, and since, because of marketing practices, this can often be done only by the consumer, the public should be warned that all vegetable products, and particularly those to be consumed raw, should be thoroughly washed with potable water. Industry should be required to follow a similar practice with raw vegetables that are to be used in compounded food products. Refrigeration serves only to minimize the spoilage of vegetables, and has little effect on any pathogens that may be present.

3.6.4 Fruit

Since most fruits are covered with a protective skin and grow above ground, they receive little contamination and seldom cause public health problems. Furthermore, the acidity of most fruits inhibits the growth of bacteria. However, fruit should be thoroughly washed before use, partic-

ularly if it is to be eaten raw. Refrigeration retards the spoilage of fruit, but has little effect on any pathogens that may be present.

3.7 Radiation Processing

The use of ionizing radiation in food processing has been under serious study for about 15 years, and research carried out in many countries is producing increasing evidence of its usefulness in the control of micro-organisms that give rise to food spoilage and those that may be potential health hazards. Irradiation has also been shown to be of value in the control of animal parasites and insects causing deterioration ; it may also be useful in the control of certain viruses found in foods of animal origin.

Most non-spore-forming pathogens and micro-organisms that cause spoilage can be easily killed by radiation doses of 0.6 Mrad or less, and pseudomonads and other cold-tolerant organisms that cause spoilage are killed by even lower doses. Such treatment can be carried out without causing any marked change in the natural characteristics of a food. Higher doses of radiation, which may be used to render a food " commercially sterile ", will kill *Clostridium botulinum* and *Cl. perfringens*. During this type of treatment, the food should be held at a temperature of 4°C or less.

Since low-dose radiation treatment can be carried out on raw products, it is believed that it can contribute to the improvement of food hygiene in the marketing system. The fact that spoilage can be retarded permits the distribution of food throughout wider areas and should, therefore, make it possible to improve the nutrition of populations in some areas.

3.7.1 Radiation sources

The sources of radiation used at present are cobalt-60, caesium-137, accelerated electrons having energies up to 10 million electron volts, and X-rays from sources of energy up to 5 million volts. It has been shown that such sources induce no radioactivity in any of the elements found in the foods of man or the feeds of animals.

These sources can be used with safety under commercial conditions and will cause no health hazard to personnel working in their vicinity, when properly shielded and monitored.

Radiation processing is advantageous for several reasons. For example, it will kill micro-organisms in foods that are in hermetically sealed packages or in the frozen state. The treatment can be carried out at room temperature without substantially raising the temperature of the product ; so-called " cold sterilization " with a radiation dose of 4.5 million rad raises the temperature of moist materials by only 10°C, a property that may be of great value in the treatment of certain foods. For example, this technique

makes it possible to kill salmonellas in blocks of frozen meat or large cans of frozen eggs without thawing these products or altering their characteristics.

3.7.2 Types of radiation treatment

Depending upon the microbiological objectives, radiation treatment can be divided into three types.[1] Definitions of the three types were given in the report of a Joint FAO/IAEA/WHO Expert Committee on the Technical Basis of Legislation on Irradiated Food,[2] and are reproduced below with slight modification.

Type I : Treatment of the food with a dose of ionizing radiation sufficient to reduce the number and/or activity of viable micro-organisms (other than viruses) to such an extent that few, if any, are detectable in the treated food by any recognized bacteriological or mycological method. No spoilage or toxicity of microbial origin is detectable no matter how long or under what conditions the food is stored after treatment, provided it is not recontaminated. The radiation dosage necessary to produce such indefinitely stable products has been tentatively placed at 4.5 Mrad, based largely upon the dosage required to inactivate *Clostridium botulinum* spores, the most radiation-resistant pathogen.

Type II : Treatment of food with a dose of ionizing radiation sufficient to reduce the number of viable specified non-spore-forming pathogenic micro-organisms (other than viruses) to such an extent that none is detectable in the treated food by any standard method. For example, salmonellas in frozen whole egg can be killed by a dosage of about 0.6 Mrad without thawing the eggs.

Type III : Treatment of food with a dose of ionizing radiation sufficient to enhance its keeping quality by substantially reducing the numbers of viable specified spoilage-causing organisms. The objective of this type of treatment is to extend the market life of a fresh product such as meat, fish, or fruit. For example, a radiation dose of about 300 000 rad increases the market life of refrigerated fish by a factor of 2–3. Such treatment markedly reduces the number of cold-tolerant organisms that can grow at the temperature of melting ice and cause the fish to become slimy.

In addition, other types of radiation treatment are used in the control of animal parasites in meat (e.g., *Cysticercus bovis* and *Trichinella spiralis*) and fish (e.g., the herring worm, *Anisakis marina*) and in the control of

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[1] The terms radappertization, radicidation, and radurization have been suggested for treatment types I, II, and III, respectively. See *Nature (Lond.)*, 1964, **204**, 237.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1966, **316** (*FAO Atomic Energy Series*, No. 6).

various viruses of animal origin (e.g., those causing foot-and-mouth disease, swine fever, and rinderpest). The radiation doses for such treatment have not been precisely worked out, but are believed to be at about 0.3–0.5 Mrad for the parasites and 2.0–4.0 Mrad for the viruses.

3.7.3 Advantages

Raw foods can be subjected to radiation treatment without marked alteration of their physical characteristics. In this respect, radiation treatment offers a distinct advantage over thermal treatment, since the temperature that kills micro-organisms causes proteins to coagulate, resulting in a semicooked product. For example, radiation treatment can be used to control the mould that causes spoilage of strawberries and to eliminate pathogens from meat, fish, and eggs, without altering the fresh, raw characteristics of these foods. A further advantage of radiation processing is the fact that it can be used to eliminate spoilage-causing organisms and pathogens from foods in hermetically sealed containers; this cannot be done with fumigating gases, steam, or dry heat without damaging the package or the food. Radiation treatment can markedly extend the market life of many products; for example, the shelf life of fresh dressed chicken held at the temperature of melting ice can be doubled by radiation treatment, which also eliminates any salmonellas that may be present. Thermal processing would result in a semicooked product.

3.7.4 Disadvantages

Enzymes are highly resistant to radiation; the normal dosage used to control micro-organisms does not inactivate them, and the quality of the product will undergo marked deterioration even though microbial spoilage may not occur. If the radiation dose is increased so as to inactivate enzymes, the product may undergo changes in flavour, colour, and texture, and with some foods a point is reached at which the adverse effect outweighs the beneficial effect. For this reason, it is not possible to produce a satisfactory sterilized raw meat product by radiation processing. However, the effects of radiation on different kinds of meat vary. For example, flavour change in mature beef is marked at high radiation doses, whereas the same doses cause no flavour change in chicken and pork. The radiation dosage to be used must, therefore, be selected in accordance with the objectives of the processing.

Viruses are resistant to radiation, and can be eliminated only by doses far greater than those that kill bacteria. For example, doses that kill salmonellas or that satisfactorily extend the market life of a food product may have little effect on viruses. If viruses are present in the food, types of treatment other than irradiation are necessary.

3.7.5 Combined heat and radiation

A combination of heat and irradiation can be used when it is not important that the fresh characteristics of a food be retained. Enzymes and viruses are, to some extent, heat-sensitive, and some of them can be inactivated by mild heating (a temperature of about 60°C). Following the heat treatment, the food can be subjected to low-dosage irradiation to extend its market life. It has been found that the effect of combined heat and irradiation is greater than that of either method used alone. The same is true of combined treatment with radiation and certain nontoxic chemicals.

4. FOODSTUFFS THAT ARE PARTICULARLY DANGEROUS

Certain foods are, for various reasons, more often involved in the transmission of disease than others. Such foods, and the factors that render them particularly dangerous, are discussed in the following sections.

4.1 Meat and Meat Products

Most outbreaks of food-borne disease in developed countries are caused by meat or meat products. Fresh, unprocessed, raw meat is rarely involved, but meat subjected to treatment such as mincing and processed foods containing meat present a greater hazard. Disease-causing organisms are only occasionally present in meat as it is obtained from animals; much more frequently, the meat is contaminated with such organisms during handling and processing subsequent to slaughter.

In developing countries, where animal diseases are more prevalent, where disease control is less efficient, and where meat inspection is not rigorous, micro-organisms derived from the animals themselves often cause human disease.

There are a number of diseases in which meat is an important source of human infection. Man may be infected either directly, as with anthrax and brucellosis, or after further development of the infective agent in an animal, as with hydatidosis. The major diseases involved are as follows : [1]

Disease in man	Principal animals involved and sources of meat infection
anthrax	cattle, buffaloes, goats, sheep, swine
botulism	all animals, environment
brucellosis	cattle, goats, sheep, swine

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[1] The list is reproduced, with slight modification, from *Wld Hlth Org. techn. Rep. Ser.*, 1962, **241**, p. 10. Diseases that are transmitted solely by contact or inhalation are not included.

Disease in man	*Principal animals involved and sources of meat infection*
Clostridium welchii gastroenteritis	all animals, environment
hydatidosis	sheep, goats, swine, cattle (cycle must be completed through dog or other carnivore)
salmonellosis	all animals, man
shigellosis	man
staphylococcal enterotoxic gastroenteritis	all animals, man
taeniasis	cattle, swine
trichinosis	swine
tuberculosis	all animals
tularaemia	hares, rabbits

Animals may be already infected with the micro-organisms that cause the above diseases, or they may be infected during the handling to which they are subjected prior to slaughter (e.g., poor transport conditions). However, procedures to which carcasses are subjected after slaughter (e.g., bleeding, jointing, evisceration, and wiping) and the use of dirty utensils are more important sources of contamination, which should be minimized by the observance of strict sanitary precautions.[1]

To prevent multiplication of contaminating micro-organisms, refrigeration is particularly important. The temperature must be sufficiently low and the cold storage rooms must be maintained at the proper degree of humidity. Carcasses should be transferred from abattoirs to retail shops or processing plants under hygienic conditions and, if possible, at low temperatures, and the same precautions must be observed in the retail shops or processing plants.

Meat wrapped in plastic must be stored at a temperature of approximately 0°C. Otherwise, depending on the gas permeability of the plastic film, preponderant development of certain species of micro-organism may occur. Since boning increases the surface area of meat, it increases the possibility of the growth of surface contaminants, including salmonellas. Boning should, therefore, be carried out under strictly hygienic conditions.

4.1.1 Minced and tenderized meat

Salmonellas are liable to grow in meat that is minced too long before it is cooked and eaten. Horse meat is particularly dangerous under such conditions. If meat is tenderized by means of appliances with blades, such appliances must be kept scrupulously clean.

4.1.2 Pork products

With raw ham, the major risk is the development of *Clostridium botulinum*. Raw dried sausages may support the massive development of entero-

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[1] See *Wld Hlth Org. techn. Rep. Ser.*, 1962, **241**.

toxic staphylococci. The most commonly occurring contaminants of cooked pork products are salmonellas and enterotoxic staphylococci. If the product has been adequately heated, such contamination will be of secondary origin (e.g., from dirty utensils, slicing machines, handlers, and sometimes wrappings). In meat products that are insufficiently cooked and then cooled too slowly, the multiplication of Clostridium perfringens, whose spores are heat-resistant, may be a problem of considerable importance.

4.1.3 Poultry

In many countries, poultry are reared in large breeding establishments, and the processing, slaughtering, and packing lines are comparable to those used in industrial abattoirs for large animals. Slaughtering, bleeding, plucking, evisceration, washing, refrigeration, and packing should be carried out with the same precautions as in large abattoirs.

The use of ready-mixed feeds for poultry has resulted in an appreciable increase in salmonella-contaminated feeds, treatment of which is essential to avoid serious risks.

The use of chlorinated water for sanitary purposes in poultry plants in some countries has brought about a substantial reduction in contamination levels.

The wide prevalence of salmonellosis in ducks makes it virtually impossible to avoid contaminated carcasses. For this reason, ducks should be thoroughly cooked before consumption.

4.1.4 Frozen meats

The quality of frozen meat is not always satisfactory, and it too often contains salmonellas. To avoid such contamination, strict precautions must be taken in all steps of production. If a low temperature is not continuously maintained, there will be a considerable increase in the number of salmonellas.

4.2 Milk and Milk Products [1]

4.2.1 Raw milk

Raw milk may be contaminated at the source (i.e., by a diseased animal) or during handling (e.g., by contact with the environment, contaminated equipment, sick handlers, or carriers).

The contaminants that present the greatest public health danger are Mycobacterium bovis, Brucella, and pathogens causing mastitis (e.g., strep-

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[1] For more detailed information, see: Wld Hlth Org. techn. Rep. Ser., 1957, 124; 1960, 197 (FAO Agricultural Studies, Nos. 40 and 52); and Abdussalam, M. et al. (1962) Milk hygiene, Geneva (World Health Organization : Monograph Series, No. 48).

tococci and staphylococci); tick-borne encephalitis virus; and *Coxiella burnetii*. Raw milk may also be contaminated with micro-organisms from human carriers (e.g., *Mycobacterium tuberculosis*, *Shigella*, and infectious hepatitis virus).

Since it may contain such a wide range of pathogens, milk is a potentially dangerous food and should undergo treatment to make it safe, even in countries where systematic campaigns are undertaken for the eradication of bovine tuberculosis and brucellosis.

4.2.2 Pasteurized milk

The objective of pasteurization is the destruction of pathogenic micro-organisms, particularly *Mycobacterium bovis* and *Brucella*.

There are several different types of pasteurization. Those of greatest importance are the low-temperature process, in which the milk is maintained at a temperature of 60-65°C for 30 minutes, and the high-temperature short-time (HTST) continuous process, in which it is held at 72°C (or a higher temperature if heavily contaminated) for 15 seconds. The HTST process is the one that is most commonly used. No matter what pasteurization procedure is used, secondary contamination must be avoided after heating, during cooling (the coolant water should be bacteriologically pure), and bottling (the equipment and containers used must be clean).

Pasteurized milk should be placed in containers which, after being sealed, should be stored at a sufficiently low temperature to prevent the multiplication of micro-organisms that may have survived pasteurization or that may have contaminated the milk subsequent to the heat treatment.

4.2.3 Sterilized milk

The milk produced by pasteurization is not sterile. Sterilization, which calls for much higher temperatures, results in a product that is safer from the public health viewpoint. Two principal sterilization processes are in use. In the VHTST (very high temperature, short time) process the milk is raised to a temperature of 130-145°C for a very short period, after which it is placed in sterile containers. In the other process, the milk is placed in glass bottles or other containers which, after being hermetically closed, are raised to a temperature of 100-120°C.

4.2.4 Condensed and dried milk

4.2.4.1 *Unsweetened condensed milk*

After concentration, unsweetened condensed milk is placed in tins that are hermetically sealed and then sterilized in the autoclave. The bacteriological properties of this product are similar to those of sterilized milk.

4.2.4.2 Sweetened condensed milk

Sweetened condensed milk is not sterilized, and its keeping properties arise solely from the initial pasteurization of the raw milk and from the high sugar concentration of the condensed product. Osmophilic micro-organisms, such as moulds, yeasts, and Micrococcaceae, can grow in such milk. Among the Micrococcaceae, the pathogenic staphylococci are dangerous, and sweetened condensed milk can contain a considerable quantity of enterotoxins.

4.2.4.3 Dried milk

Dried milk is produced by concentrating the milk and then dehydrating it, either by passage over hot rollers or, more commonly, by spray drying. During concentration of the milk, staphylococci may produce enterotoxin. In the course of spray drying, the temperature at the centre of the milk droplets remains at 60–80°C for only a short time, and bacteria that are susceptible to heat can survive.

4.2.5 Fermented milk

Fermented milk products include yoghourt (yaourt), kefir, and soured milk products made with Lactobacillus acidophilus. The micro-organisms responsible for fermentation greatly lower the pH, making multiplication of pathogenic bacteria impossible. However, such pathogens are not destroyed, and preliminary pasteurization of the raw milk used to prepare these products is advisable.

4.2.6 Butter

Two types of butter are available : farm butter, which is prepared from raw cream, and pasteurized butter, which is prepared from pasteurized cream and seeded with selected lactic ferments. Farm butter is potentially dangerous, since it contains any pathogenic bacteria that may have been present in the milk from which it was made. Pasteurized butter presents no health risk if hygienic precautions are taken during its manufacture.

4.2.7 Cheese

Cheese is made by curdling milk with rennet, lactic ferments, or both. To prevent its contamination by pathogenic bacteria, the following precautions are essential :

(1) whenever possible, it should be made from pasteurized milk ;

(2) the lactic acid fermentation should be as rapid as possible, to limit the multiplication of pathogens ;

(3) the bacteriological quality of the rennet should be checked ; and

(4) all possible hygienic precautions should be taken during manufacture.

The following types of cheese are subject to dangerous contamination :

(1) Cream cheese sometimes contains *Salmonella*, staphylococcal enterotoxin, or tick-borne encephalitis virus.

(2) Soft or semihard cheese made from goat's, cow's, or ewe's milk may be contaminated with *Brucella*.

(3) Hard cheese sometimes contains botulin.

(4) Processed cheese may contain spores of *Clostridium perfringens* and *Cl. botulinum* that can germinate, particularly when the product is packed in hermetically closed containers.

4.3 Prepared and Ready-to-Eat Nonsterile Foods

Technological advances have made available throughout the world a great variety of prepared or " convenience " food items. All such foods are characterized by their simplicity of preparation for eating. Although they are not sterilized during production, they usually receive a heat treatment of some type, the purpose of which may be to cook the food, to inactivate enzymes, or to kill undesirable micro-organisms. However, such heat treatment does not eliminate spore-formers and other relatively resistant micro-organisms.

Prepared and ready-to-eat products may be classified according to their composition and method of preservation, as follows. (Certain products, particularly those in groups 1 and 2, may be handled during preparation and packaging, and are susceptible to recontamination from food handlers, unclean equipment, or contaminated raw materials.)

(1) Ready-to-eat meat, sausage, poultry, fish, and salad. All these foods are heat treated during preparation, most contain a small amount of salt, and some are smoked for flavouring purposes. Most must be refrigerated to prevent the growth of spoilage-causing organisms, salmonellas, and toxigenic bacteria. A hazard of botulism has been recognized with certain types of lightly salted, highly moist smoked fish.

(2) Cooked, ready-to-eat meat dishes, seafoods, desserts, potatoes, and complete meals. These products are prepared in final form and then frozen for preservation. The cooking process should destroy all harmful organisms except spore-formers, but hazards may develop if the products are not protected from recontamination before packaging and freezing. .One potential hazard is straphylococci originating from food handlers. Special care must be taken to avoid inadvertent thawing and refreezing of these products because of the hazard of botulin or staphylococcal toxin formation.

(3) Instant dry foods. Milk, coffee, tea, soups, and potatoes are available in forms that permit rapid rehydation and consumption without cooking. Unless precautions are taken, such products may become contaminated during and after drying. Milk, in particular, has been shown to be susceptible to contamination with salmonellas during processing.

(4) Dehydrated cake mixes, puddings, and custards. These products usually require some degree of cooking but it is not always sufficient to destroy salmonellas and other pathogens that may be introduced by the addition of contaminated eggs to the dry mix.

(5) Mayonnaise and salad dressings. Salmonellas may enter these products through contaminated ingredients such as eggs and egg products, but they apparently die rapidly if the pH is below 4.5. It has been shown that products (e.g., potato salad) prepared with mayonnaise can transmit salmonellas if they are prepared from contaminated ingredients and are insufficiently acidic.

Attention must be paid to the bacteriological quality of the ingredients of all compounded foods. For example, it has been shown that candy can be contaminated with salmonellas through the addition of carmine red. This food colour, extracted from the cochineal beetle, has been found to harbour *Salmonella cubana*.

Since prepared, ready-to-eat foods are not cooked before consumption, it is generally considered that the meeting of microbiological standards is more important for them than for other foods. In many of these products, the presence of large numbers of bacteria suggests that they have been handled or improperly stored or cooked inadequately.

4.4 Raw Products

Foods that are normally eaten raw present a health hazard if they are contaminated with pathogenic micro-organisms. Fruits and vegetables that grow in or near the ground (e.g., strawberries) may become contaminated with viruses and enteric pathogens if irrigated with polluted water or fertilized with human or animal excreta. Similarly, shellfish from polluted waters may carry the viruses that cause infectious hepatitis, poliomyelitis, and possibly other enteroviruses, as well as pathogenic enterobacteria. Eggs frequently carry salmonellas and, if consumed raw or undercooked, may cause disease. Serious outbreaks of salmonellosis in hospitals have been attributed to the use of cracked eggs in patients' diets.

4.5 Other Foods

Foods other than those listed in the preceding sections may carry pathogenic micro-organisms or their toxic metabolites and thereby con-

stitute a hazard to public health. For example, dried or frozen eggs often contain salmonellas. Pasteurization of liquid eggs before processing is an effective preventive measure, provided recontamination is avoided. Salmonellas have also been troublesome in shredded coconut and dried yeast, which they contaminate during or after processing.

Certain fish products distributed in the USA have been shown to carry a botulism hazard—e.g., certain types of fish from the Great Lakes, where *Cl. botulinum* type E is known to be indigenous. Such fish is lightly salted by soaking in brine, then cooked and smoked in a hot air oven; the product, which is not usually cooked before consumption, is relatively moist and often contains little salt. Spores of *Cl. botulinum* type E, if present on the raw fish, can survive the heat treatment and, if the product is not adequately refrigerated, may germinate and produce botulin. A salt concentration of about 3.5% in the aqueous phase of the muscle of the fish is sufficient to prevent toxin formation.

Pickled fish also present a botulism hazard if the pH is not below 4.5. Similarly, home-made cucumber pickles have, on rare occasions, been found to contain botulin when the salt and acid content were insufficient to prevent the growth of *Cl. botulinum*.

4.6 Food Handling at the Consumer Level

Since many foods, including processed foods, may not be free from pathogenic micro-organisms, all consumers should be informed of the dangers involved. Cooks, housewives, and all others who prepare food should be aware of the necessity for proper refrigeration and cooking and of the dangers of cross-contamination. Such information can be made available by means of television, radio, and newspapers and other publications. It is important that it be explained in simple terms, so that it will be easily understood by the public. The Committee considers that, in the present circumstances, it is of the utmost importance to provide such information to the public.

However, those who prepare such educational material should be aware of the danger of frightening the consumer about certain specific food products.

5. ROLE OF THE LABORATORY IN FOOD HYGIENE PROGRAMMES

The food hygiene laboratory is indispensable in a food hygiene programme. It has three principal fields of activity, as follows.

(1) It performs microbiological examinations of foods and of clinical material, and conducts epidemiological investigations of outbreaks of food-borne disease.

(2) It conducts continual surveillance so as to collect information on the microbiological status of foods circulated within the country. Such surveillance must be based on collaboration between the field and the laboratory services, and should include the regular control of imported foods and those intended for export. Before a food can be assessed microbiologically, complete information is necessary on its source, the ways in which it has been processed and handled, and on technological and environmental factors that may have affected it.

(3) Based upon the information gathered by the activities noted above, it traces the sources of contamination of foods and undertakes studies of ways to correct the situation.

The food hygiene laboratory is often asked to furnish information about the microbiological status of foods in terms of their freedom from pathogens. Certain regulations and laws require that food " shall be free from any pathogens capable of causing human disease ". However, the food hygiene laboratory is in no position to certify a food as being pathogen-free. If pathogens are not found in a food, it is not necessarily true that the food does not contain them ; the result simply means that none was found by the methods used. In view of the varying sensitivity of microbiological tests and of the fact that they can reveal the presence of only a limited range of pathogens, it is important that foods not be certified as being free from such organisms, since such certification might lead to the impression that the foods concerned were so safe that subsequent care in handling could be disregarded. The Committee recommends that no microbiological results be reported without a qualifying statement indicating the number of samples examined from a given batch of food, the amount of each sample, and the methods used. The results should be reported in terms of the laboratory's being unable to find any pathogens in the food, rather than in terms of the food's containing no pathogens. This practice may be difficult to institute and may be objected to by administrators. However, results issued without such qualifications are not complete.

5.1 Administration and Training

Divided authority, and differences of purpose and of professional background among laboratory personnel, have often led to unsatisfactory conditions, characterized by a lack of co-ordination and uniformity. In any programme for the development and operation of food hygiene laboratories, due consideration should be given to achieving a co-ordinated administration and to the proper training of all staff members.

5.2 Sampling and Laboratory Methods

Techniques of sampling and laboratory examination must, of course, be selected in accordance with the desired objectives. In investigating

outbreaks of food-borne disease, all available foods should be sampled. Similarly, all possible methods of laboratory examination should be used, so as to minimize the possibility of overlooking the presence of pathogens. Random sampling of a given food product would usually be adequate in surveillance programmes, whereas sampling based upon statistical evaluation would be necessary in deciding whether to accept or reject a given consignment of food (e.g., for import or export). Assessment of the changing microbiological condition of foods by means of sampling at different stages of processing is a useful procedure.

It is most important that standardized methods be used for both sampling and laboratory analysis. Standardization should take place at three levels : within each laboratory, throughout all the laboratories in a given country, and at the international level. Various international, governmental, and other bodies have done a considerable amount of work in this area, and have published recommendations for standardized microbiological procedures that have been selected after comparative tests in different countries.[1]

5.3 Microbiological Standards for Foods

The principal reason for examining foods microbiologically and for establishing microbiological standards for foods is to determine their safety for consumption. At present there are no microbiological standards that cover all the factors that may cause food to be injurous to health. At most, the existing standards cover micro-organisms that may cause infectious disease or form toxic compounds in food ; viruses, animal parasites, toxic chemicals, and fungal toxins are not covered.

5.3.1 Types of standard

Two types of bacteriological standard are available : (1) standards for specific types of pathogen and (2) standards based on total bacteria counts or on the enumeration of certain indicator organisms such as Enterobacteriaceae, *Escherichia coli*, and enterococci.

Methods for the determination of pathogens and toxins in foods cover organisms such as *Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, *Cl. botulinum*, *Bacillus cereus*, and *Vibrio parahaemolyticus*. Such methods are useful both in epidemiological investigation and in surveillance programmes ; however, if all of them are carried out on a certain food with negative results, there is no assurance that other—perhaps more important—pathogens are not present.

The enumeration of microbial populations indicates whether or not excessive bacterial growth has taken place in a food as a result of con-

[1] For further information, consult the works listed in the Bibliography, Annex 9

tamination, improper storage, or both. It may indicate whether the food has come into contact with faecal material or been recontaminated after processing. Satisfactory results of such enumeration may indicate that some of the conditions that make food safe have been fulfilled, but give no assurance of the absence of pathogenic agents.

A combination of both types of bacteriological standard is preferable ; in many instances it would offer valuable information on the microbiological status of a food product, although not an absolute guarantee of safety.

5.3.1.1 *Standards for particular foods*

No bacteriological standard is applicable to all kinds of food, since each type of food presents special problems.

Generally speaking, standards for raw foods offer little or no assurance that such foods are safe for consumption. Nevertheless the use of a specific standard for a certain pathogen (e.g., *Salmonella*) is valuable in any corrective programme undertaken when a particular food has been identified as an important source of that pathogen.

Bacteriological standards are a valid means of measuring the safety of sterilized foods and the efficiency of their processing.

The use of bacteriological standards for processed but not sterilized foods offers varying degrees of assurance of safety, depending upon the type of food and the way it is processed. For example, excellent methods are available for the testing of precooked frozen foods.[1] Both enzyme tests and microbiological tests should be performed to measure the efficiency of processing (e.g., to test the adequacy of pasteurization of milk and eggs).

5.3.2 Evaluation of standards

In view of the wide variations in food practices and in basic food needs that prevail in different countries, it does not seem practicable to set a single rigid standard for each food. If standards are to be set, that which is desirable and that which is attainable with sound practices must be carefully evaluated. The paramount consideration is the welfare of the people, and such standards should serve to improve the hygienic quality of their food.

In considering the application of bacteriological standards for foods to international trade, the economic implications of internationally agreed standards should be carefully weighed against the benefits that they could be expected to have for human health.

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[1] See : American Public Health Association (1958) *Recommended methods for the microbiological examination of foods*, New York.

5.3.3 Legal and administrative standards

It is advisable to adjust bacteriological standards periodically, so as to stimulate progressive improvement in the quality of food. Owing to the complexity of the factors involved, the Committee recommends that such standards be formulated mainly for administrative or advisory use. It further recommends that legal bacteriological standards should be restricted to carefully selected foods where they serve a clearly defined purpose.

6. RESEARCH NEEDS

The Committee endorses the recommendations for research made by the IAMS International Committee on Microbiological Specifications for Foods,[1] which are reproduced below with slight modification and additions.

Salmonella

(a) Determination of the basic causes of the varying precision of a given method for the isolation of salmonellas when it is used for different types of food (e.g., dried and frozen egg products, cured meat products, raw sausages, poultry, " red " meats, precooked frozen foods, and powdered milk, all of which may exhibit specific peculiarities).

(b) Development, for epidemiological purposes, of specific phages for the typing of important serotypes of *Salmonella* in addition to *S. typhi*, *S. paratyphi*, *S. typhimurium*, and *S. thompson*.

(c) Development of standard phages for *Salmonella* typing in sufficient quantities to permit their international distribution, together with their propagating host strains.

Clostridium botulinum *

(a) Development of rapid methods for detecting toxins of each type in foods by either *in vivo* or *in vitro* tests.

(b) Development of methods and media for isolating *Cl. botulinum* from foods, including enrichment media and selective or differential media.

(c) Study of the factors leading to the accumulation of *Cl. botulinum* type E in nature.

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[1] International Association of Microbiological Societies, International Committee on Microbiological Specifications for Foods (1967) *The significance of microorganisms in foods* . . (mimeographed report).

* Sections marked with an asterisk have been added by the present Committee.

Clostridium perfringens and Bacillus cereus

(a) Determination of whether or not *Cl. perfringens* food-poisoning is caused by serologically identifiable types of this organism.

(b) Determination of the nature of the specific substance or substances produced by *C. perfringens* and *B. cereus* that cause food-poisoning.

Staphylococcus

(a) Determination of the total number of enterotoxins produced by staphylococci and of their serological specificity, and development of rapid methods for determining the amounts of such enterotoxins in foods and for extracting them from foods.

(b) Development of simple, specific methods for the enumeration of the following strains of *S. aureus* in foods : coagulase-positive strains, enterotoxigenic strains, and strains pathogenic for man.

(c) Determination of the significance of the coagulase test as an indicator of the production of enterotoxins by staphylococci, in relation to clotting-time and degree of coagulation.

(d) Determination of the effect of commensal micro-organisms on the production of staphylococcal enterotoxins, covering the induction of toxin formation, the amounts produced, and activation and/or inactivation.

(e) Study of the environmental and nutritional factors conducive to the production of staphylococcal enterotoxins.

(f) Resolution of the conflicting findings on the transduction by phage of toxinogenesis by staphylococcal strains.

Other micro-organisms

(a) Verification of the significance of the total Enterobacteriaceae count as an indicator of objectionable enteric contamination.

(b) Clarification of the role of the enterococci as food-poisoning agents and of their significance as indicators of insanitary practice, and clarification * of the role of *Arizona*, enteropathogenic *Escherichia*, *Klebsiella*, *Citrobacter*, and *Hafnia* in outbreaks of food-borne disease.

(c) Development of methods for the recovery from foods of pathogens normally considered rare in countries with well developed food technology (e.g., El Tor vibrio and *Vibrio cholerae*).

(d) * Study of the occurrence of *Mycobacterium avium* in food and clarification of its importance in outbreaks of food-borne disease.

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* Sections marked with an asterisk have been added by the present Committee.

Viruses

(*a*) Study of the range of viruses pathogenic to man that may contaminate foods.

(*b*) Study of the persistence of given viruses in foods.

(*c*) Determination of the specific D-values for the destruction of food-borne viruses by heat and by gamma radiation.

(*d*) Development of methods for the detection of viruses in foods.

(*e*) Epidemiological studies of the role of foods as vehicles for the transmission of viral disease.

(*f*) Determination of whether oncogenic viruses occur in foods.

Mycotoxic fungi

(*a*) Further studies of the significance of mycotoxins for human health.

(*b*) A search for mycotoxins other than those now known to exist (i.e., aflatoxins, ochratoxin, patulin, maltoryzine, islanditoxin, " ATA ", stachybotrys toxin, etc.).

(*c*) Study of the distribution of mycotoxic fungi and their toxins in foods.

(*d*) Study of the environmental factors that are conducive to the elaboration of mycotoxins.

Miscellaneous

(*a*) Development of polyvalent fluorescent-antibody sera that can be used to detect the presence of the following organisms in food : salmonellas ; *Clostridium botulinum* types A–F (both cells and toxins) ; and, if possible, strains of *Clostridium perfringens* that cause food-poisoning.

(*b*) Statistical studies of the bacterial content of processed foods in relation to the standards of sanitation in food factories.

(*c*) Statistical studies to permit the application of rational sampling schemes to the microbiological analysis of given types of food.

(*d*) * Improved methods of microbiological examination for use in the surveillance of slaughterhouses, processing plants, and food dispensing establishments.

Annex 1

PRINCIPAL FEATURES OF FOOD-BORNE DISEASES

Causal agent	Delay in onset of symptoms after eating	Main symptoms
Chemical, irritant [1]	10 min–2 h	nausea and abdominal pain, then vomiting and diarrhoea
Staphylococcus sp. (enterotoxin) [2]	1–6 h ; usually 2–4 h	severe nausea, cramps, vomiting, diarrhoea, prostration ; subnormal temperature, lowered blood pressure
Salmonella sp. [2]	6–48 h ; usually 12–24 h	abdominal pain, diarrhoea, frequent vomiting ; fever nearly always present
Clostridium perfringens [2]	8–22 h ; usually 10–12 h	abdominal pain, diarrhoea
"Non specific" bacteria and Bacillus cereus [1]	3–18 h	diarrhoea, abdominal pain, vomiting
Vibrio parahaemolyticus [3]	at least 8 h ; usually 15–17 h ; rarely 18–24 h	abdominal pain (mainly epigastralgia), diarrhoea (usually watery), vomiting, fever ; rarely headache and chills
Clostridium botulinum [2]	12–36 h ; rarely up to several days	change in voice, such as hoarseness ; vomiting, diarrhoea, weakness, dizziness, headache, constipation ; oculo-motor or other cranial nerve paralysis
Chemical, neurotoxic [1]	short (e.g., sodium fluoride)	muscular paresis
	10–12 days (e.g., o-tricresyl phosphate	flaccid paralysis
Trichinella spiralis [2]	2–28 days ; usually about 9 days	diarrhoea, abdominal pain ; oedema of eyelids and face ; subconjunctival, subungual and retinal haemorrhage ; muscle soreness and pain ; skin lesions, thirst, profuse sweating, chills, weakness, prostration ; eosinophilia ; fever is usual

[1] Data from : Great Britain, Ministry of Health (1958) *Food poisoning*, London.

[2] Data from: American Public Health Association (1965) *Control of communicable diseases in man*, 10th ed., New York.

[3] Data from : Aiso, K. & Matsumo, M. (1961) *Jap. J. Microbiol.*, **5**, 338.

Annex 2

SPECIMEN FORMS FOR THE REPORTING
OF FOOD-BORNE DISEASE OUTBREAKS *

Case History

NAME OF PERSON		ADDRESS	
OCCUPATION	AGE	SEX	TELEPHONE No.

Did individual partake of the suspected meal ? ☐ yes   no ☐
If yes, date and hour food eaten :
Did individual become ill ? ☐ yes   no ☐
If yes, date and hour of onset :
Below is a list of foods and beverages served at the suspect meal or consumed 72 hours prior to onset (check only those which were eaten).
Date .... Date .... Date ....

FOOD ITEM		FOOD ITEM		FOOD ITEM	
Breakfast *a*					
Lunch *a*					
Dinner *a*					

a Indicate place eaten.

Which of the following symptoms did the individual have, and how long (in hours) did they last ?
Nausea or Vomiting.............. Diarrhoea.................... Fever ....
Abdominal Cramps
and Pain .... Paralysis.................... Bloody Stools ....
Other ....
Was a physician consulted ? ☐ yes ☐ no   Diagnosis :
If yes, physician's name and address :
Name of hospital, if hospitalized :
Names of other persons who partook of the suspect meal :
....

....
Date   Investigator

Annex 2 (*continued*)

Food Preparation and Sanitation Report
(Food-Borne Disease Outbreak)

1. OUTBREAK AT (*name and address of establishment*) — DATE OF OUTBREAK — TIME OF SUSPECTED MEAL

DAY AND HOUR OF ONSET	FIRST CASE	LAST CASE	NUMBER OF PERSONS EXPOSED	NUMBER OF PERSONS ILL

2. FOOD HISTORY

Food item	Source	Preparation of item			Storage and refrigeration	Results of laboratory examinations
		When ?	Where ?	By whom ?		

3. CANNED FOODS (If commercially prepared, identify manufacturer, brand, and lot number. If home canned, describe process used) :

Annex 2 (*continued*)

4. HISTORY OF FOOD HANDLERS :

Name	How long employed here ?	Duties	History of recent illness	Laboratory examination

5. SANITATION (INCLUDING WATER, PLUMBING, ETC.)

....

....

....

6. EPIDEMIOLOGICAL CONCLUSIONS :

   a. Suspected (or confirmed) food items :

   b. Causative agent :

   c. How was food contaminated ? :

Date investigation begun	Date of this report	Name(s) of investigator(s)

NOTE : Completed regular inspection form and narrative report should be attached.

Annex 2 (*continued*)

Summary of Case Histories

LOCATION OF OUTBREAK (*name and address of establishment*)

DATE OF OUTBREAK

Names of persons (sick or well) who consumed suspected food or drink	Age	Ill (yes or no)	Required medical aid (yes or no)	Date and hour food eaten	Date and hour of onset of illness	Incubation period [a]	Food served at suspected meal (check foods eaten)							Symptoms							
														Nausea or vomiting	Diarrhoea	Fever	Abdominal cramps and pain	Paralysis	Bloody stools	Other

SUSPECTED FOOD OR FOODS (*Include origin of each food item*) : ....

DATE OF REPORT : ....   INVESTIGATOR : ....

[a] Interval of time between ingestion of food and onset of illness.

Annex 3

PROCEDURE FOR COLLECTING AND SUBMITTING FOOD SAMPLES FOR LABORATORY EXAMINATION *

Samples of food suspected of causing outbreaks of food-borne disease are often received in nonsterile containers wrapped in newspaper or other unsuitable coverings, and are not refrigerated. Consequently, they are partly or wholly decomposed. They are sometimes received with no identification other than the sender's name, and with no information other than a statement that the food had caused such an outbreak.

Food-borne outbreaks may be the subject of court proceedings, and laboratory personnel are called upon to testify as to their analysis of the food. Such testimony obviously is of little or no value when the record of the sample is incomplete, and laboratory results are of no significance if the sample is received in a nonsterile container or in a state of decomposition. Therefore, the following procedures should be followed :

Notification of laboratory

Since laboratory analyses of food samples require some preparatory work, it is important that the laboratory director be given advance notice, preferably by telephone or wire, of the number and types of samples being submitted. If possible the laboratory should be contacted prior to collection of specimens for suggestions on type and size of specimens to collect, refrigeration, method of shipment, and other details.

Asepsis

Aseptic technique must be followed in preparing the sample. Knives, spatulas, or other instruments used to obtain a sample must have been previously sterilized in an open flame or by using one of the methods described below. Foods in small closed or covered containers should not be removed from the container, but should be sampled in their entirety.

Sample containers

Bulk samples must be placed in sterile containers. Sterile water-sample bottles, which may be obtained from the local water company or health department, are ideal for liquids, semisolids, or shredded material. A wide-

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* Source : see Annex 2.

mouth jar or pail with a tight-fitting lid may be used for large samples, provided it is thoroughly cleaned and sterilized. A local hospital or laboratory may be able to furnish a sterile container, or to sterilize containers which are to be used. If containers are to be sterilized, it is recommended that steam under pressure—exposure at 121°C (250°F) under pressure of 15 pounds for 20 minutes—be used. If this method of sterilization is not possible, dry heat at 160°C (320°F) for 4 hours, or 170°C for 1 hour, or boiling water—complete immersion for 30 minutes—may be used. A statement of the method used to sterilize the container must appear on the sample collection form.

Identification

The sample must be properly identified. Proper identification would include the place and time the sample was collected, the method of collection if indicated, the reason it is being submitted for analysis, the organism or chemical suspected, and any other pertinent information.

Sealing

The sample must be sealed. Gummed paper tape, surgical adhesive tape, or plain paper covered with cellophane tape may be used to seal the sample container. The date and time of sealing and the name of person who collected and sealed the sample should be written on the tape. The seal should be affixed to the inner food container in such a manner that the container cannot be opened without breaking the seal.

Accompanying data

The sample should be accompanied by as much information as may be immediately available, such as (a) the number of persons who may have become ill, (b) the elapsed time between ingestion of the food and onset of symptoms, (c) the symptoms observed or reported, and (d) the reason for suspecting this food. An accurate statement of the symptoms experienced, particularly the incubation period, is especially valuable as a guide in the conduct of laboratory tests.

Refrigeration

Perishable samples that are collected in the non-frozen state must be refrigerated from the time they are collected until they are received at the laboratory. They should be maintained at a temperature of 0–4.4°C (32–40°F), but should not be frozen. Samples not immediately sent to the laboratory for analysis should be retained under refrigeration for possible

later study. All perishable samples should be refrigerated until they are either sealed in their original containers or placed in sterile containers. The sealed sample container should be enclosed in an outer container (preferably insulated) with sufficient refrigerant to provide refrigeration during transit. Outer containers of polystyrene (expanded styrene) with cans of frozen water as refrigerant usually provide adequate refrigeration. Dry ice should not be used for nonfrozen specimens. When samples must be sent to the laboratory by public conveyance or mail, it must be remembered that they may be delayed in transit. Therefore, sufficient refrigerant should be used so that some will present when the sample is opened. (Refrigerant must not come in direct contact with the food sample.) The container must be prominently labelled : " Perishable Food Sample for Bacteriological Examination—Rush ", and should be sent by the fastest possible means of transportation. Samples so labelled should receive immediate attention, regardless of their time of arrival.

Samples of frozen foods should be maintained in the frozen state by the use of dry ice until they are delivered to the laboratory.

Interpretation of laboratory results

The interpretation of laboratory examinations is very important, and sometimes difficult. In some instances the degree of positiveness is more important than the mere fact that the specimen is positive. Consultation may be obtained from the laboratory director, epidemiologist, or health officer.

Sample Collection Report

DATE	CITY	SAMPLE NUMBER

Description of sample and source :

....

....

Identifying marks on seal (e.g., code or lot number)

Name of person from whom sample collected

Name and address of establishment where collected

....

Name of dealer, manufacturer, or processer, if known

....

Analysis requested (chemical, bacteriological, etc.)

....

Method of sterilizing container

....

Method of sterilizing utensils used to collect samples

Other suspected foods :

....

....

....

....

Method of shipping sample (air, rail, etc.)

....

....

INVESTIGATOR ....

Laboratory results :

....

....

....

....

....

....
Date of laboratory report   Laboratory investigator

....

Name and address of laboratory

Annex 4

FOOD-BORNE DISEASE OUTBREAKS OF ALL TYPES
IN ENGLAND AND WALES, 1961-65 *

Presumed causal agent	1961	1962	1963	1964	1965
Salmonella typhimurium	2 503	1 866	1 820	1 725	1 721
Other salmonellas	1 268	982	1 149	1 368	1 224
Staphylococci	91	143	74	107	74
Clostridium welchii (perfringens)	83	84	82	85	64
Other agents	1 442	1 448	1 340	1 087	1 008
Total incidents	5 387	4 521	4 465	4 372	4 091
Cases	12 750	9 696	13 104	9 975	11 317
Deaths	22	23	27	19	19

* Data compiled from annual reports published in the *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service*.

Annex 5

LABORATORY DETECTION OF CLOSTRIDIUM BOTULINUM
AND ITS TOXIN

Clostridium botulinum in food, soil, fish, bottom sediments, and similar materials is usually detected by inoculating suitable enrichment media and, after a suitable incubation period, testing for toxin by injection into mice. To minimize interference from other organisms, the original sample may be heated to 80°C for 10 minutes if the presence of *Cl. botulinum* type A or B is suspected; however, this treatment cannot be used with type E because of the thermal sensitivity of its spores.

Toxin appearing in the enrichment culture can be identified by conventional mouse-protection tests with type-specific *Cl. botulinum* antisera. The

[1] For further information, see : Ingram, M. & Roberts, T. A., ed. (1967) *Botulismus : Proceedings of the Fifth International Symposium on Food Microbiology, Moscow, 1966*, London, Chapman & Hall; and Lewis, K. H. & Cassel, K., ed. (1964) *Botulism* : *Proceedings of a Symposium, Cincinnati, 13–15 January*, Cincinnati, Ohio, Public Health Service (Publication No. 999-FP-1).

organism may be isolated by streaking the culture onto blood agar, egg yolk agar, or other suitable media and picking characteristic colonies.

To examine a food suspected as being the cause of a botulism outbreak, an extract of the product is injected into mice with and without type-specific antisera. It is essential to determine the type of toxin as quickly as possible, so that antitoxin can be promptly administered to the patient.

Methods for the detection of *Cl. botulinum* and its toxin in natural materials are not standardized, and work is in progress on simpler and quicker methods, such as the fluorescent antibody technique.

Annex 6

STAPHYLOCOCCAL ENTEROTOXIN

In the investigation of suspected staphylococcal poisoning outbreaks, it is common practice to examine suspected foods for coagulase-positive staphylococci. Numbers in the millions per gram are suggestive, but do not give definitive proof that the food contains enterotoxin ; similarly, low numbers of staphylococci do not necessarily prove that the food is safe. For example, outbreaks of poisoning have resulted from the consumption of food containing few or no viable staphylococci ; staphylococci may grow in a product at an early stage of processing and be killed by heat (e.g., in dried milk) or die during storage (e.g., in cheese).

Definitive evidence that a specific food is involved in an outbreak of poisoning can be provided only by the demonstration of enterotoxin. A method based on extraction and concentration of the toxin followed by a gel-diffusion test with specific antisera has been described,[1] but it is too complicated for general use and a simple sensitive test is needed.

There is a serious lack of information on the thermostability of the enterotoxins, although preliminary information suggests that crude toxin may be more stable than the purified product. Recent reports indicate that if canned vegetables and similar products with low acidity are heated at 125°C for 30–90 min, staphylococcal enterotoxin is inactivated. However, many canned foods are heated much less adequately, and staphylococcal enterotoxin, if present, may survive in such foods. Thus, although ordinary cooking procedures destroy staphylococci, they cannot be relied upon to inactivate the toxin.

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[1] Casman, E. P. (1958) *Publ. Hlth. Rep. (Wash.)*, **73**, 599.

Annex 7

IDENTIFICATION OF VIBRIO PARAHAEMOLYTICUS [1]

Vibrio parahaemolyticus is a Gram-negative, monoflagellated short rod. Faecal specimens to be tested for the presence of the organism should be obtained at as early a stage of illness as possible and should not be chilled. Selective media have been developed for the culture of *V. parahaemolyticus*. To identify the organism, the following biochemical tests should be performed : (1) fermentation of glucose, lactose, and sucrose (sucrose and lactose are not fermented, and only acid is produced from glucose) ; (2) hydrogen sulfide production ; and (3) the Voges-Proskauer test. Tests of halophilic properties should also be carried out. *V. parahaemolyticus* is moderately halophilic, growing abundantly in a medium of pH 5.0–9.0 containing 2–3% sodium chloride. It grows in peptone medium if the sodium chloride concentration is 7%, but not if it is 10%.

More precise identification may be achieved by agglutination tests. Three antigenic components—O, K, and H—have been recognized in *V. parahaemolyticus,* and 10 O-antigens and 32 K-antigens have been classified. H-antigen determination is difficult because of the single flagellum ; particular types of K-antigen are found only with particular types of O-antigen. However, agglutination tests may be performed with pooled K-antisera.

Annex 8

MYCOTOXINS IN FOODS [2]

The Committee considered the large volume of evidence relating to toxin production by various mould species and their incidence in human foodstuffs. Several of these species have been clearly implicated in incidents of toxicity in man, for instance *Claviceps purpurea* in ergotism and several species, e.g., *Fusarium poae*, *Fusarium sporotrichicides* and *Cladosporium epiphyllum*, in alimentary toxic aleukia. Numerous other moulds which can develop in a wide variety of foodstuffs have been shown capable of producing substances toxic to various animal species. Outstanding are *Penicillium islandicum*, a species primarily involved in toxic yellow rice,

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[1] For further information, see : Fujino, T. et al. (1953) *Med. J. Osaka Univ.*, **4**, 299 ; Sakazaki, R. (1967) *Isolation and identification of Vibrio parahaemolyticus.* In : Fujino, T. & Fukumi, H., ed. *Vibrio parahaemolyticus*, Tokyo, Naya Shoten.

[2] Reproduced from *Wld Hlth Org. techn. Rep. Ser.*, 1967, 377, p. 55 (*FAO Nutrition Meetings Report Series*, 1967, No. 42).

which produces the toxins islanditoxin and luteoskyrin, and strains of *Aspergillus flavus* which produce a group of closely related chemical substances known as the aflatoxins. These latter substances were initially isolated and identified from a batch of groundnut meal which caused deaths in turkeys. Since this initial discovery it has been found that aflatoxin can arise in a number of other foodstuffs if they stand long enough at temperature and moisture levels favourable to the growth of moulds. They have even been found occasionally in foods which are potential sources of protein concentrates for use in infant feeding, such as cottonseed, and in staple foods such as maize.

In view of the fact that aflatoxin may be present in components of children's formulae (groundnuts in particular being a valuable and, in many regions, a readily available protein ingredient) and in view of the importance of minimizing exposure of children to such agents, the Committee reviewed the report of the meeting of the FAO/WHO/UNICEF Protein Advisory Group in August 1966 which had made recommendations on this subject. On the best evidence available, derived from experiments conducted on various animal species including primates, this Group recommended that a level of aflatoxin in protein supplements, of which 100 g is eaten per day, should not exceed 0.03 ppm (30 μg/kg) of food. " Although the Group would prefer to impose a lower level of aflatoxin in the foods and food mixtures concerned in order to provide a wider margin of safety, it believed that there was an even more urgent need to provide extra protein in some parts of the world in order to prevent malnutrition and starvation. These considerations outweighed the desirability of introducing measures for reducing a hypothetical health hazard by limitations which were difficult to enforce under current agricultural practices and techniques of food processing." The Group hoped that with further improvements in agricultural practices it would become possible " to insist on lower levels of aflatoxin in food and yet remain confident that adequate supplies of protein-rich foods would remain available ". The Committee endorsed this statement (although ideally it too would have preferred aflatoxin-free food) and recognized the maximum level of aflatoxin which had been specified by the Protein Advisory Group as set out above. Some specified level is needed for routine food testing ; nevertheless, the Committee emphasized the tentative nature of these recommendations and the need for further research which should include even longer term feeding trials than have so far been possible.

The Committee drew attention to the number of test methods for aflatoxin currently in use and which have been published [1] and also noted

[1] Campbell, A. D. & Funkhouser, J. I. (1966) *J. Ass. off. agr. Chem.*, **49**, 730 ; Coomes, T. et al. (1965) *Analyst*, **90**, 492 ; Iongh, H. de et al. (1964) *Vet. Rec.*, **76**, 901 ; Lee, W. V. (1965) *Analyst*, **90**, 305 ; Nabney, J. & Nesbitt, B. (1965) *Analyst*, **90**, 155 ; Pons, W. & Goldblatt, L. A. (1965) *J. Amer. Oil Chem. Soc.*, **42**, 471.

that the International Union of Pure and Applied Chemistry is currently engaged in an international collaborative study of these tests with a view to producing an agreed standard test.

Mould toxin production, whether it occurs in oilseeds, cereals or other foodstuffs, can be minimized by careful control of harvesting, handling, processing (particularly drying), and storage methods. The Committee emphasized the need to educate and train all those concerned with the production, distribution and marketing of foodstuffs in the importance of avoiding conditions which favor mould growth.

Annex 9

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